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Morphometric and Electrophoretic Separation of Four Populations of *Archachatina marginata* in Nigeria.

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Abstract
Morphometric analysis of shell variations and hepatopancreas protein electrophoresis of Archachatina marginata in two vegetation zones of rainforest and derived savanna of Nigeria were performed with a view to determining whether the species understudy can be separated into subspecies or not. The snails were sampled from four locations representing two vegetation zones: the rainforest (Ile-Ife, Okitipupa and Calabar) and derived savanna of Nigeria (Lokoja). The shell of each snail was described by six measurements using Vernier calipers immediately they were brought into the laboratory. Principal Component Analysis (PCA) and Canonical Variates Analysis (CVA) were performed on the morphometric data using PAST software while the protein profile of the four populations was performed by using Sodium Dodecyl Sulphate-Polyacrylamide gel (SDS-PAGE). Both the PCA and CVA produced non-overlapping clusters indicating separation of some samples into distinct sub populations. Height of shell which was the most variable was the most suitable for delimiting the populations. The hepatopancreas protein profile of the four populations of Archachatina marginata does not reveal any significant separation. This study concluded that multivariate morphometric analysis of shell measurements can be useful in separating A. marginata into sub-populations while the protein profile does not separate the populations.

***Keywords**: **Archachatina marginata*, Cluster analysis, Morphometric, Protein profile, Polymorphism, Phenotypic plasticity, Sub-species.**

Introduction

The African giant land snail *Archachatina marginata* which belongs to the molluscan Family Achatinidae is widespread in the rainforest zone of Nigeria. The animal, along with other achatinid snails are served as delicacies, as the meat is highly nutritive, contains high protein, iron, phosphorus, but low in sodium and cholesterol (Akinnusi, 2002; Ejidike, 2002).

The much varied and remarkable polymorphism exhibited by the shell pattern of this species makes the use of conchological features inadequate and unreliable in the delimitation of the *Archachatina* species into different subspecies. Several workers have employed measurement of shell parameters to determine variations in snails. Moneva *et al*., 2012 applied geometric morphometrics to distinguish sexual dimorphism and shape of *Vivipara angularis* while

Sobrepena & Demayo (2014) studied the banding pattern and shell shape morphology of *Achatina fulica* in fifteen different provinces in the Philippines. In Nigeria, few studies have been carried out on the taxonomy of the achatinid snails. These include Banjoko (2010), where measurements of shell parameters of *A. marginata* were used to ascertain the degree of relationship between the parameters. Awodiran *et al*., 2012 reported that the multivariate analysis of the morphometric measurements of shells of *A. marginata* of three vegetation zones in Nigeria did not produce a definite clustering pattern. In a study carried out by Awodiran *et al*. (2013) on the protein profile of five species of achatinid snails (*A. marginata, Achatina achatina, Achatina fulica, Archachatina papyracea* and an unidentified land snail) from some parts of southern and north-central states of Nigeria to determine genetic similarity between them and also to show whether eight populations of *A. marginata* contained sub populations, it was reported that cluster analysis of the *A. marginata* populations produced two sub species which showed geographical separation of the species.

In this present study, an attempt is made to combine the use of protein electrophoresis with analysis of measurements of shell parameters to delimit four populations of *A. marginata* from two different vegetation zones of Nigeria.

Materials and methods

Sample collection and locations

A total of one hundred and twenty six (126) matured snail specimens of *A. marginata* were sampled from four locations representing two vegetation zones: the rainforest and derived savanna of Nigeria. The maturity of the snail specimens was determined by examining whether the ovotestis is fully developed or not and also by counting the number of whorls which is five in matured snail specimen. The rainforest locations included Ile-Ife, Okitipupa and Calabar while the derived savanna was Lokoja. The map of the study area showing the sampling sites (with map of Nigeria inset) is shown in Fig.1 while information on different numbers of sample per population is given in Table 1. The vegetation varied from rainforest to savanna (Happold, 1987). The annual rainfall ranges in between 1,000mm to 2500mm. (Adegbola & Onayinka, 1976; Salako, 2007).The identification of the land snails was carried out using keys prepared by Bequaert (1950) and Mead (1995). Four snails per population were used for protein electrophoresis.

Shell measurement

The shell of each snail was described by six measurements using Vernier calipers immediately they were brought into the laboratory. The shell characteristics measured include the following: height of shell (HS), width of shell (WS), spire length (SL), 1st whorl length (1WL), aperture height (AH), and aperture width (AW) as recommended by Madec & Bellido, (2007) (Fig. 2). The height of shell (HS) was measured using Vernier calipers from A to B while the width of shell (WS) was measured from point B to C. This was similarly performed for all other shell characteristics.

Statistical analysis

A multivariate Cluster Analysis was carried out on the morphometric data obtained to illustrate patterns of correlation among populations from various localities (Madec *et al*., 2003; Chiu *et al*., 2002). 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).

Homogenization of tissue
Homogenization of the same weight (1.5g) of hepatopancreas of the snails was carried out in 0.2M Phosphate buffer (pH 7.0) containing 0.15M NaCl. The homogenate was centrifuged at 25,000 rpm. The clear supernatant was removed and used as the crude protein sources.

Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis (SDS-PAGE).
Disc gel electrophoresis in the presence of Sodium Dodecyl Sulphate was performed on the various samples on 7.5 % separating gel and 4.5% stacking gel as described by Weber and Osborn (1975) using the SDS-Tris-glycine buffer system at pH 8.8. A volume of 0.1ml of the sample was added to 0.9ml of sample buffer (0.01M Tris-HCl, 1% SDS, 1% β-mercaptoethanol, pH 7.2) and boiled for 5 mins in a boiling water bath. It was then allowed to cool to room temperature. Glycerol (2 drops) and 5 µl of tracking dye (0.2% Bromophenol blue) were added to the boiled sample. 50 µl of the sample solution was layered on the stacking gel. Electrophoresis was carried out at room temperature at a constant current of 1 mA/gel during stacking and 3 mA/gel during actual separation. The gels were stained in the staining solution made up of Coomassie Brilliant Blue R-250 (1.25g), 227ml methanol, 46 ml glacial acetic acid and distilled water to make a total volume of 500 ml. The gels were destained in the destaining solution which is 5% methanol and 7.5 % glacial acetic acid in distilled water.

Protein data analysis
The protein banding patterns of the four populations studied were scored (manually for presence or absence of bands). Only reproducible and valuable bands were scored. The data were subjected to Jaccard cluster algorithm using the statistical software PAST. This was employed to determine the similarity coefficient of the protein banding patterns.

Results

Morphometric studies
The various locations sampled are illustrated in Fig. 1 while the sample size and the geographical coordinates are listed in Table 1. The average values, ranges and standard errors for all the shell variables measured are shown in Table 2. Wide range of sizes especially on height shell were found in the samples of Ile-Ife and Okitipupa (Height of Shell SD 1.23 and 0.96, respectively), revealing great heterogeneity in the populations. The standard deviations for all the Height of Shell for all the populations were found to be higher than other parameters measured.

Fig. 3 shows the principal component analyses of *A. marginata* shell measurements from all the locations of study. All the clusters produced by the Principal components analysis (PCA) did not overlap; Lokoja though overlap with Calabar is disparate from both Okitipupa and Ile-Ife. However, the Ile-Ife population overlaps with Okitipupa. Canonical variates analyses (CVA) were conducted to see if the population of *A. marginata* encountered in the sampling could be separated intraspecifically are shown in Fig. 4. The CVA plots produce the same result as the PCA. From Fig. 5, HS is the character most responsible for variation among the various clusters of the snails from the various ecological zones (loading, 0.7157), while, to a significantly lesser magnitude, AH and WS have the second (0.4179) and third (0.3634) heaviest loadings. Data for

these characters were also compared in a box plot (Fig. 6). These box plots represent summaries of measurements of the three highest loading characters (HS, AH and WS) in the principal components analysis. Subjected to Student's t-test, these measurements are not significantly different for both aperture height and width of shell.

Table 1: Locations and sample sizes (n) for *Archachatina marginata*

Zone	Locality	Geographical coordinates	Sample size (n)
Rainforest	Ife	7°28'N 4°34'E	33
	Okitipupa	8°30'N 4°33'E	31
	Calabar	4°57′N 8°19′E	31
Guinea savanna	Lokoja	9°05'N 7°32'E '	31
Total			126

Table 2: The Range (R), Mean (X) and Standard Deviation (SD) of each Morphometric variable of *Archachatina marginata* from different locations.

Variables		Ile-Ife	Okitipupa	Calabar	Lokoja
N		33	31	31	31
HS	R	11 – 17	10.7 – 14.3	8.7 – 11.9	9.3 – 12.3
	X	14.12	12.59	10.12	10.68
	SD	1.23	0.96	0.73	0.78
WS	R	6.2 – 8.6	5.8 – 7.4	4.5 – 6.1	4.9 – 6.2
	X	7.36	6.64	5.26	5.38
	SD	0.58	0.45	0.41	0.31
SL	R	4.2 – 7.7	4.3 – 6.6	3.9 – 5.7	4.0 – 6.5
	X	6.27	5.70	4.51	5.10
	SD	0.78	0.58	0.43	0.58
IWL	R	1.9 – 3.9	2.1 – 3.3	1.8 – 2.4	2.0 – 3.7
	X	3.06	2.69	2.08	2.54
	SD	0.43	0.34	0.16	0.50
AH	R	7.0 – 9.8	7.0 – 8.7	5.2 – 6.8	5.7 – 7.1
	X	8.24	7.82	5.84	6.23
	SD	0.59	0.54	0.43	0.38
AW	R	3.6 – 5.4	3.6 – 4.6	2.9 – 5.6	3.0 – 4.5
	X	4.70	4.07	3.43	3.43
	SD	0.47	0.27	0.49	0.35

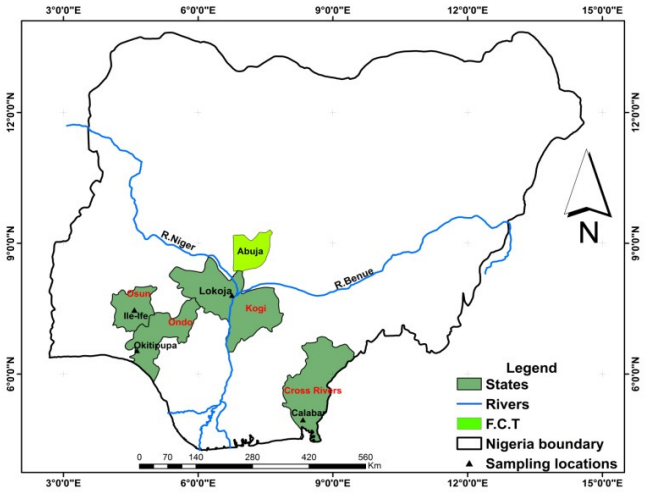

Fig.1: Map of *Archachatina marginata* sampling locations of Ile-Ife, Okitipupa, Calabar and Lokoja.

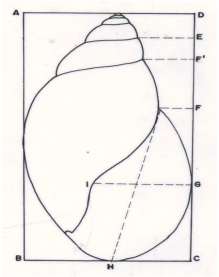

Fig 2: Measurements of shell morphology used in multivariate analyses (AB=Height of shell, HS; BC= Width of shell, WS; DF=Spire length, SL; EF^1=1^{st} whorl length, 1WL; FH=Aperture height, AH and GI=Apertue width, AW.

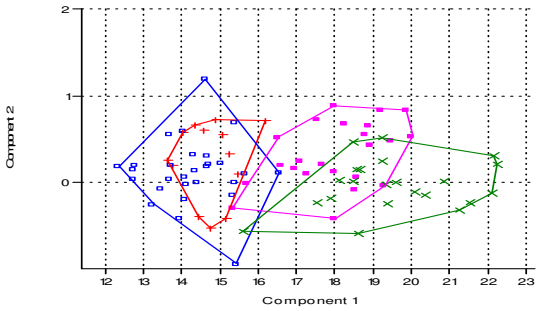

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Fig. 3: Principal components analyses based on shell measurements of *Archachatina marginata* specimens from Lokoja (red +), Calabar (blue □), Okitipupa (purple ■), Ile-Ife (green x)

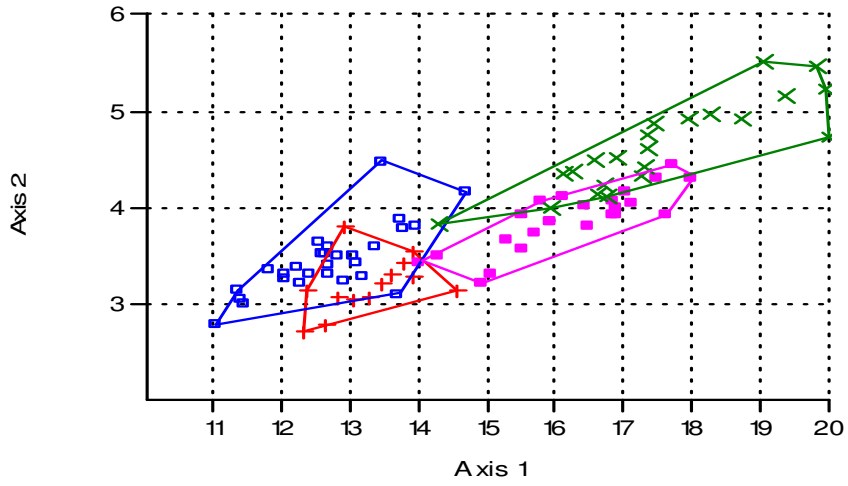

Fig 4: Canonical variates analyses based on shell measurements of *Archachatina marginata* specimens from Lokoja (red +), Calabar (blue □), Okitipupa (purple ■), Ile-Ife (green x).

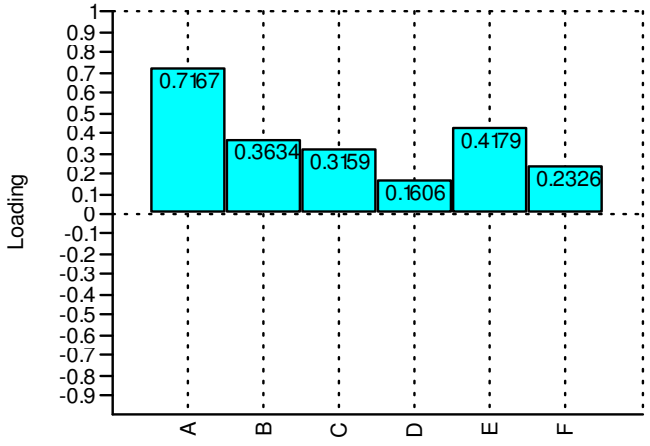

Fig. 5: Respective snail shell characters and their loadings on PC1 of the principal components analysis (A=HS, B=WS, SL=C, 1WL=D, AH=E, AW=F).

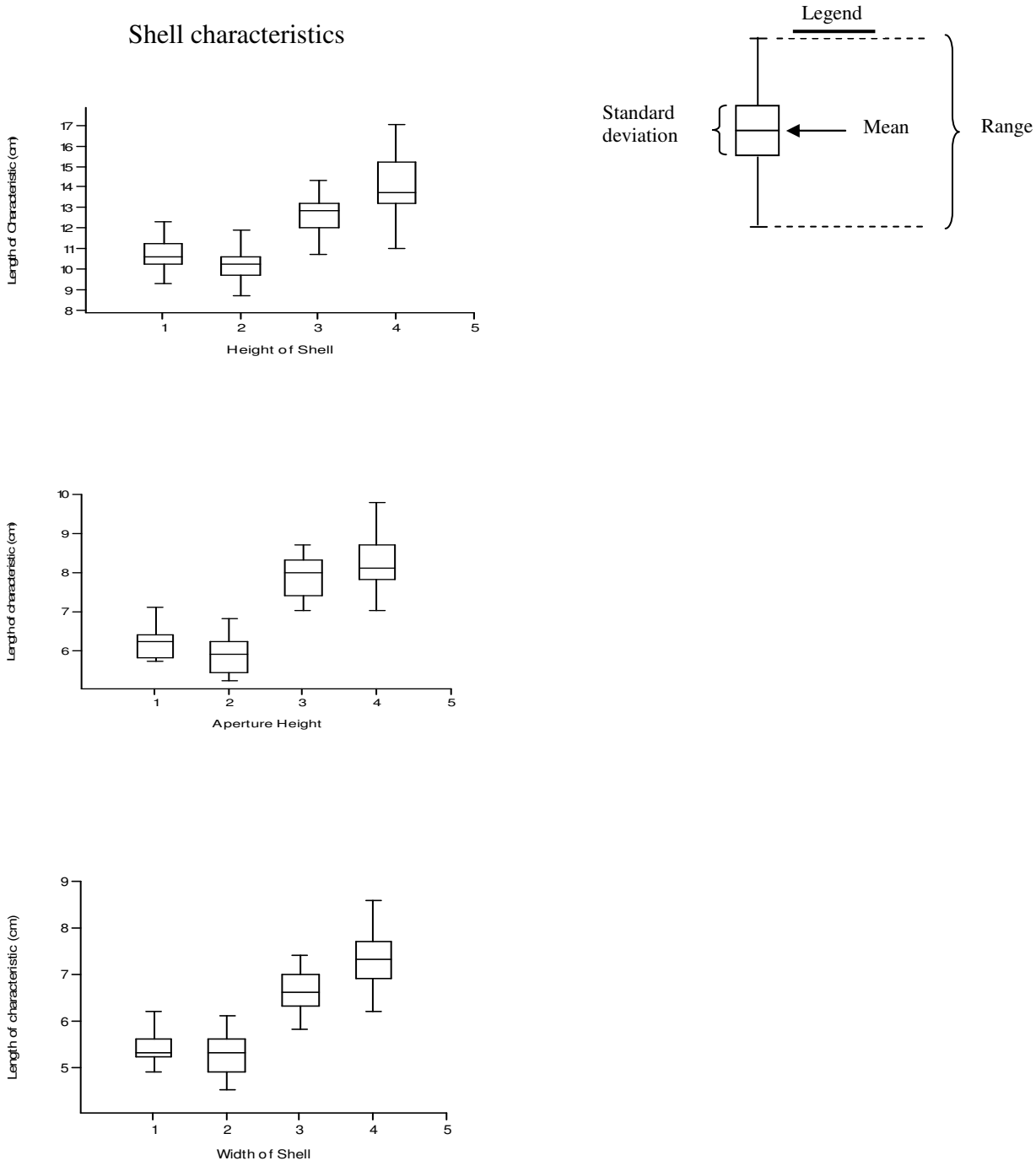

Fig 6: Box plots of morphometric characteristics (mean ± std, dev.) for *Archachatina marginata* from four different locations (Ile-Ife, Okitipupa, Calabar, Lokoja) for three different characteristics Height of shell, Aperture Height and Width of Shell.

Protein Studies

Fig. 7 and Fig. 8 show protein banding patterns of the four different populations of species *A. marginata*. An examination of these patterns reveals distinct quantitative and qualitative intra specific variation in terms of position and intensity of bands.

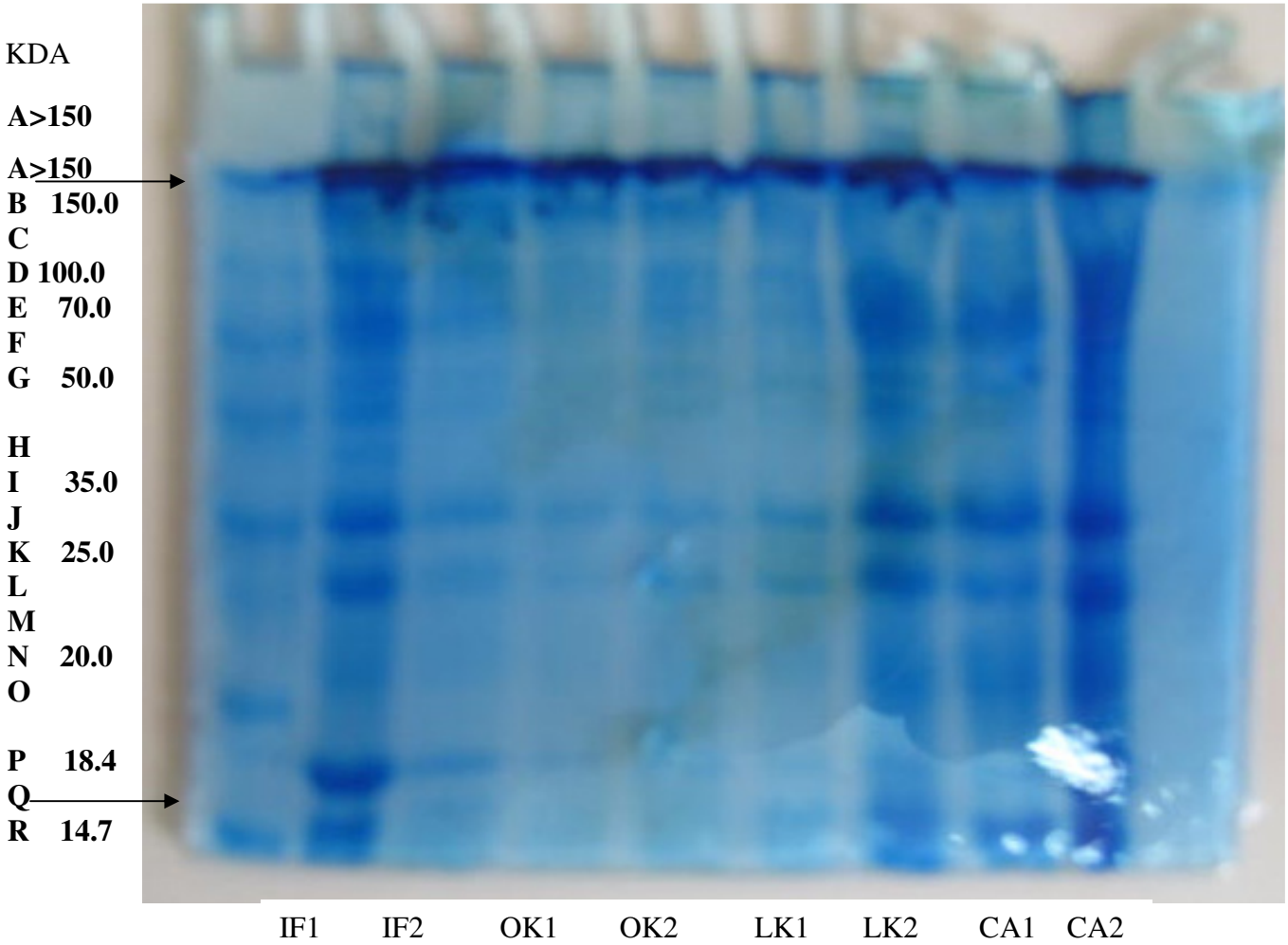

Fig 7: A typical Coomassie-Blue stained SDS-PAGE gel showing banding patterns for each of the twelve populations. (IF1- ILE-IFE 1, IF2- ILE-IFE 2, OK1- OKITIPUPA 1, OK2- OKITIPUPA 2, LKI- LOKOJA 1, LK2- LOKOJA 2, CA 1- CALABAR 1, CA2- CALABAR 2).

The protein banding patterns revealed sixty eight bands across all samples. Fig. 8 shows the clustering patterns in the dendrogram based on protein banding pattern data of all the populations sampled *marginata*. The cluster also shows two clades. This means that there is a very high level of genetic heterogeneity within the various samples of *A. marginata*. The bands also had molecular weights ranging from 14.7 to 150 KDA. Most individuals are indistinguishable and the two clusters present do not really manifest separation into distinct sub-groups.

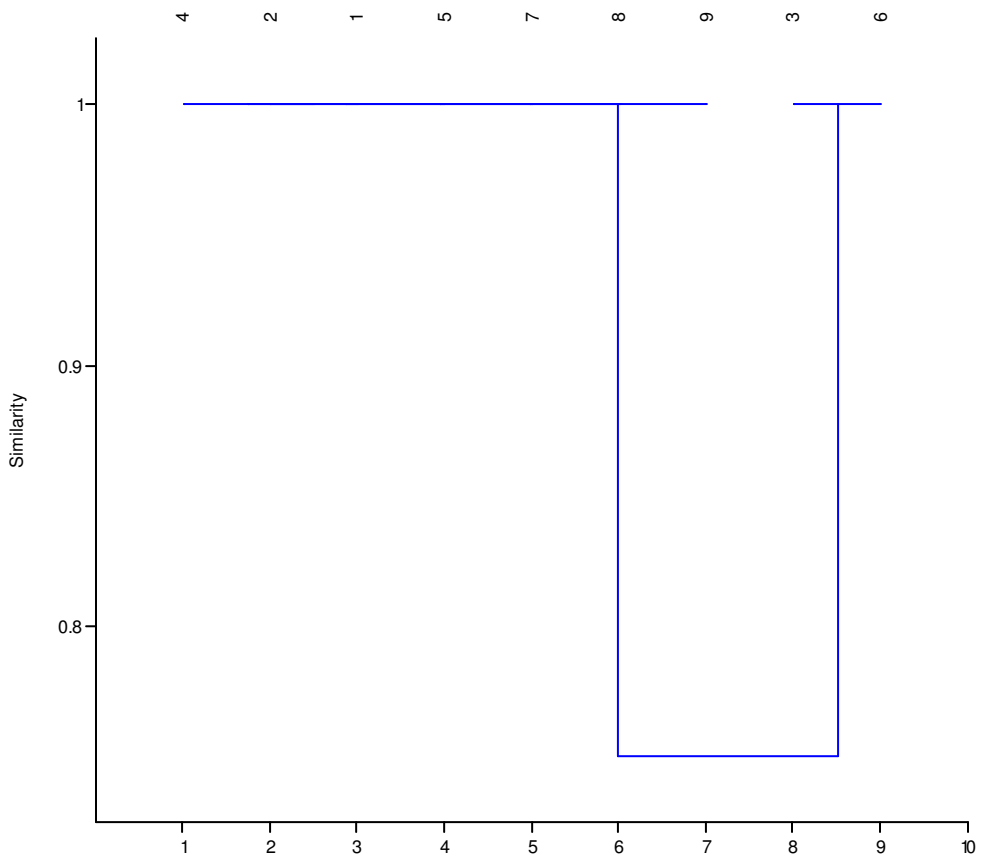

Fig. 8: Dendrogram showing similarity coefficients of the four populations of *Archachatina marginata* (Paired group Jaccard similarity measure).

Discussion

For the principal components analyses on morphometric parameters of the snails, PC1 shows separation of two major groups. One group consists of the Calabar and Lokoja clusters, while the other group is made up of the Okitipupa and Ile-Ife clusters. This separation along PC1 is congruent with geographical location, as Okitipupa and Ile-Ife are situated relatively closely in southwestern Nigeria within the forest zone. On the other hand, Lokoja lies in north central Nigeria at the confluence of the Niger and Benue rivers, while Calabar is located in south southern Nigeria. In other words, the Principal Component Analysis on the morphometric parameters of the specimens of *A. marginata* of two different populations of Calabar and Lokoja produced clusters which overlapped. There was also an overlap of the two clusters of Okitipupa and Ile-Ife. The lack of separation between clusters of Okitipupa and Ile-Ife may be due to the geographical proximity of the two populations. They are both based in rainforest zone of the south western Nigeria. The difference in vegetation zones of Calabar (lowland rainforest) and Lokoja (Guinea savanna) could not produce separate clusters of their populations.

The cluster of the population of specimens from Lokoja was found to be separate from that of Okitipupa and Ile-Ife while that of Calabar did not overlap with Ile-Ife. This shows that samples from north central ecological zone represented by Lokoja were disparate from those of the south western zone which was represented by Okitipupa and Ile-Ife.
It was also observed that intraspecific differences of the shell parameters were significant enough to separate the specimens across ecological zones into sub clusters which may indicate sub populations.

The Canonical Variates Analysis (CVA) also showed the same pattern of clustering. There was considerable separation of the samples into distinct sub populations. Among the shell parameters measured, the height of shell (HS) was the most variable and consequently the most suitable for the separation of the populations. The aperture height (AH) was also significant as a diagnostic characteristic in separating the populations.

Most snail specimens' show striking phenotypic plasticity (Madec and Bellido, 2007) and both evolutionary pressures and local environmental factors have been found to play a role in the variation of shell morphology. Dupouy *et al.* (1993) also opined that ecological factors may affect shell morphology through phenotypic plasticity or through local natural selection. In the study carried out by Awodiran *et al*. (2012), there was an overlap among the samples of populations of *A. marginata* of three ecological zones in Nigeria. However, the samples from Lokoja consistently showed the same pattern as in the present study.

This present study on the hepatopancreas protein profile of four populations of *A. marginata* does not reveal any significant pattern of clustering along the four different sampling locations. This implies that the populations are the same, although there is high genetic heterogeneity between the populations. This does not conform to the results on the morphological study. Earlier work by Awodiran *et al*. (2013) reported very high genetic differentiation among eight populations of *A. marginata* in Nigeria which showed a great potential of delimitation to sub species. The use of total protein gel electrophoresis method in separating different populations of the same species has been reported to be limited since not all nucleotide substitutions may lead to changes detectable by the technique (Mahmoud, 2012). In other words, the protein gel electrophoresis of the present work is not congruous with the polymorphic nature of the shell pattern studied. Therefore to get a better resolution of the genetic variation among the populations of the species studied there is need to employ the use of DNA sequencing.

In conclusion, the morphometric analysis of four populations of *A. marginata* across both rainforest and Guinea savanna zones of Nigeria produced at least two different clusters of the species studied while the deployment of gel electrophoresis of its hepatopancreas protein did not show electrophoretic pattern of any taxonomic usefulness.

References

Akinnusi, O. (2002) Introduction to snail and snail farming. Triola Publishing Company, Abeokuta. pp.70.

Awodiran, M. O., A. O. Olayemi, and J. I. Awopetu (2012) Morphometric studies of land snails, *A. marginata* (Swainson, 1821) in some South-West, South-South and North Central States of Nigeria. *International Journal of Academic Research*. 4. (6):

Awodiran, M.O., J. I. Awopetu, O.O. Odekanyin and D.O. Ogunbosoye (2013) An electrophoretic study of protein diversity in five species of land snails (Achatinidae) in Nigeria. *International Journal of Biological and Chemical Sciences* 7(1): 86-95.

Adegbola, A.A. and E.A.O. Onayinka (1976) A review of range management problems in the Southern guinea and derived savanna zones of Nigeria. *Tropical Grasslands* 10 (1): 41-51.

Banjoko, O.A. (2010) Estimation of surface area of the giant African land snail (*Archachatina marginata* and relationship to internal shell volume. B. (Agric.) Thesis University of Nigeria. 39pp.

Bequaert, J.C. (1950) Studies in the Achatininae, a group of African land snails. *Bulletin of Museum and. Comparative Zoology 105:*1-126.

Chiu, Y., H. Chen, S. Lee and C.A. Chen (2002) Morphometric Analysis of Shell and Operculum variations in the viviparid snail, *Cipangopaludina chinensis* (Mollusca: Gastropoda), in Taiwan. *Zoological Studies* 41 (3): 321-331.

Dupouy, J. (1993) Correspondence analysis of shell morphology in the African Freshwater snail *Biomphalaria pfeifferi* (Kraus 1848) (Pulmonata: Gastropoda). *Biological Journal of Linnaean Society* 50: 329-338.

Ejidike, B.N (2002) Snail rearing practices in Southern Nigeria. Proceedings of the 27th Annual Conference of Nigeria Society for Animal Production (NSAP) March 17-21, 2002. Akure, Nigeria. pp. 307-308.

Hammer Øyvind, D.A.T. Harper and P.D. Ryan (2006) PAST –Palaeontological Statistics- version1.58. http://folk. Uio.no/ohammer/past.

Happold, D.C.D. (1987) The mammals of Nigeria. Clarendon Press, Oxford. 402pp.

Madec, L., A. Bellido and A. Guiller (2003) Shell shape of the land snail *Cornu aspersum* in North Africa: unexpected evidence of a phylogeographical splitting. *Heredity*.91: 224-231.

Madec, L. and A. Bellido (2007) Spatial variation of Shell morphometrics in the sub antartic land snail *Notodiscus hookeri* from Crozet and Kerguelen Islands. *Polar Biology* 30: 1571-1578, *Springer Verlag.*

Mahmoud, M.A.M., S.S. El-Akad, S.M. ,Zalat and S.A. Awni (2010) Polymorphism in the Protein of the thoracic muscle of an endemic bee (*Anthophora pauperata*) in the St. Katherine Protectorate. *Egyptian Journal of Biology* 12: 6-11.

Mead, A.R. (1995). Anatomical studies reveal new phylogenetic interpretations in *Lissachatina* (Pulmonata: Achatinidae). *Journal of Molluscan studies* 61: 257-273.

Moneva, C.S.O., Torres, M.A.J.; Demayo, C.G. (2012). Applications of geometric morphometric analysis in describing sexual dimorphism in shell shapes in *Vivipara anguillaris* Muller (Family Viviparidae). *Animal Biology & Animal Husbandry* 4(1): 14-18.

Salako, F.K. (2007). Temporal variation of rainfall erosivity in Southern Nigeria. Asset Series A. 7: 190-202.

Sobrepena, J.M.M.; C.G. Demayo (2014). Banding pattern and shape morphology variations in shells of the invasive African land snail *Achatina fulica* (Bowdich, 1822) from the Philippines. *Annals Biological of Research* 5(1): 64-79.