



## Colour Polymorphism as an Indicator of Population Genetic Structure in *Archachatina marginata* from Southern Nigeria

Etukudo, O. M.<sup>1\*</sup>, Ekerette, E. E.<sup>2</sup>, Job, I. E.<sup>2</sup>, Unah, U. L.<sup>3</sup> and Bassey, N. S.<sup>1</sup>

### Abstract

The present study was designed to investigate the phenomenon of color polymorphism in the genetic structure of the African snail species *Archachatina marginata* from Akwa Ibom, Cross River, and Rivers States in Nigeria. For this purpose, 48 adult snails were collected and analyzed, including 24 from each ecotype: 24 black-skinned (BS) and 24 white-skinned (WS), with 16 from each state. Genetic diversity was evaluated using mitochondrial cytochrome b and Single Nucleotide Polymorphism (SNP) markers. WS ecotypes were found to have formed a cohesive group with minimal genetic divergence (0.04-0.10), suggesting recent maternal ancestry and high gene flow. On the other hand, BS ecotypes were found to have high genetic diversity with certain lineages (CYT\_12 and CYT\_15) showing deep divergence (0.45-0.63). Genetic divergence between WS and BS ecotypes was found to be moderate (0.470). Phylogenetic tree results showed that the mitochondrial DNA formed three major clusters with mixed color morphs. Haplotype diversity was found to be high in both ecotypes: BS (1.000) and WS (0.968), while nucleotide diversity was found to be moderate. These findings suggest that colour polymorphism in *A. marginata* snails is actually a phenotypic variation in a genetically linked population rather than genetic ecotypes. However, the limitations of the study include the low number of samples and the use of only mitochondrial markers. Future studies should include a larger population and geographic distribution of the samples and use nuclear markers to reveal the genetic basis and evolutionary implications of colour polymorphism in *A. marginata* snails.

✉ Etukudo, O. M.: [owoism1981@gmail.com](mailto:owoism1981@gmail.com)

<sup>1</sup>Department of Biological Sciences, Faculty of Computing and Applied Sciences, Topfaith University Mkpatak, Akwa Ibom State, Nigeria.

<sup>2</sup>Department of Genetics and Biotechnology, University of Calabar, Cross River State, Nigeria.

<sup>3</sup>Department of Animal Science, Faculty of Agriculture, University of Uyo, Uyo, Nigeria.

**Keywords:** Colour polymorphism; *Archachatina marginata*; Single Nucleotide Polymorphism (SNP); Mitochondrial DNA; Genetic diversity; Phylogenetic analysis; Gene flow.

### Article history

Received: January 6, 2026

Received in revised form: March 4, 2026

Accepted for publication: March 7, 2026

Available online: March 14, 2026

### 1.0 Introduction

Giant African land snails are hermaphroditic mollusks belonging to the family Achatinidae,

which includes about thirteen genera. Common species found in Nigeria and other African countries include *Archachatina marginata*,

*Achatina achatina*, *Achatina fulica*, *Thapsia* species, and *Limicolaria* species (Okon & Ibom, 2012; Etukudo *et al.*, 2018). These snails thrive in warm climates with mild year-round temperatures and high humidity. They are believed to have originated in the coastal regions of Tanzania and Kenya, spreading across sub-Saharan Africa and other continents through human-mediated transportation. Most populations are concentrated in West and East Africa (Okon *et al.*, 2012; Odeh *et al.*, 2015), and they have been introduced elsewhere for use in folk medicine and as a food source (Odeh *et al.*, 2015).

Giant African land snails exhibit variation in skin colour, which may reflect species identity, environmental conditions, or habitat differences. Typical colours include brown (Fig. 1), black (Fig. 2), and white/albino (Fig. 3). The foot is usually smooth but may appear slightly rough or wrinkled when dry. While body colour is genetically determined, environmental factors such as diet and habitat can influence its intensity and variation. Some colour patterns also provide camouflage against predators. Variations within natural populations have been studied extensively (Okon & Ibom, 2012; Ibom *et al.*, 2016; Etukudo, 2017; Etukudo & Okon, 2025).

Colour polymorphism (the co-occurrence of two or more distinct colour morphs within a population), is a well-recognized biological phenomenon with important ecological and evolutionary implications. Colour differences often reflect underlying genetic variation generated by mutation, natural selection, genetic drift, and gene flow. For this reason, colour polymorphism is frequently used as a phenotypic marker to study population genetic structure and evolutionary processes in species with limited molecular resources (Galeotti *et al.*, 2003; McKinnon & Pierotti, 2010).

Giant African land snails also exhibit variation in shell colour, banding, and body pigmentation, sometimes forming distinct morphotypes within the

same geographic population. These variations can result from genetic polymorphism, environmental factors, or their interaction, making these snails a useful model to explore the relationship between phenotypic variation and population differentiation.



**Figure 1:** Brown-skinned snail (*A. marginata*)



**Figure 2:** Black-skinned snail (*A. marginata*)



**Figure 3:** White-skinned snail (*A. marginata*)

It is vital to understand the relationship between color morphs of the giant African land snails, whether it is the result of genetic differentiation or environmental variation, to comprehend the evolutionary mechanisms of diversity maintenance within the populations of the giant African land snails (Eze *et al.*, 2010; Okon and Ibom, 2012; Okon *et al.*, 2013; Inemotimineri *et al.*, 2025).

The study of colour polymorphism as a marker of population genetic structure is particularly relevant for giant African land snails due to their limited dispersal ability, localized breeding populations, and susceptibility to habitat fragmentation. These factors may promote

genetic differentiation, potentially leading to the emergence of new morphotypes associated with specific ecological niches. By integrating colour polymorphism with molecular or genetic analyses, it is possible to assess how phenotypic variation correlates with gene flow, reproductive isolation, and local adaptation (Galeotti *et al.*, 2003; McKinnon and Pierotti, 2010).

Despite the significance of color polymorphism in *A. marginata*, few studies have established a direct relationship between the visual expression of colour morphs and genetic differentiation using molecular markers. Most of the previous research has been conducted using morphological traits and a few molecular markers, and this has created a void in the understanding of the relationship between color polymorphism and population structure, gene flow, and reproductive connectivity. There is a lack of SNP and mitochondrial markers in Nigerian snail populations, which creates a void in understanding the evolutionary mechanism of color diversity.

This study, therefore, aims to investigate the population genetic structure of black-skinned and white-skinned *A. marginata* snails in Nigeria using SNP markers as a tool to quantify genetic differentiation, gene flow, and haplotype diversity across geographic regions.

## 2.0 Materials and Methods

### 2.1 Study area

The research was carried out in the Biotechnology Laboratory of Topfaith University, Mkpatak, Akwa Ibom State, Nigeria.

### 2.2 Experimental animals

A total of 48 adult *Archachatina marginata* snails were used for this study. These were made up of 16 snails from each of the three states: Akwa Ibom, Cross River, and Rivers States, Nigeria. These were comprised of 24 black-skinned ectotype snails acquired from local markets and 24 white-skinned ectotype snails acquired from their natural habitats, as they are not commonly sold in local markets.

### 2.3 Tissue collection

Snails were euthanized by mechanically breaking the shell to extract the soft tissue from the shell and visceral fluid. A section of the muscular foot was dissected under sterile conditions and used for subsequent DNA extraction.

### 2.4 DNA extraction

Genomic DNA was extracted from the foot tissue using a modified CTAB protocol (Gupta, 2019). The tissue samples were ground in liquid nitrogen, and 20 µl of proteinase K (10 mg/ml) was added. The mixture was incubated at 60°C for 10 minutes. DNA was purified using phenol: chloroform: isoamyl alcohol (25:24:1), precipitated with cold isopropanol, washed with 70% ethanol, and resuspended in 100–200 µl of TE buffer. RNase treatment was applied to remove RNA contaminants. DNA concentration was adjusted to 30–50 ng/µl and stored at –20°C.

### 2.5 DNA quantification

DNA quality and integrity were assessed using 1.5% agarose gel electrophoresis with a 100 bp DNA ladder. Gels were stained with ethidium bromide and visualized under UV light (470 nm Blue LED Portable Gel Hood, Royal Biotech/ Biolympics).

### 2.6 SNP marker genotyping

The snail samples were analyzed for single nucleotide polymorphisms (SNPs). SNP genotyping was performed using a PCR–Restriction Fragment Length Polymorphism (PCR-RFLP) approach targeting the mitochondrial cytochrome b (CYTB) gene. The CYTB gene fragment was amplified using primers:

Forward (F): 5'- CCATCCAACATCTCAGCATGATGAAA-3'

Reverse (R): 5'-CCCCTCAGAATGATATTTGTCTCA-3'

PCR amplification conditions followed standard protocols, and amplified products were verified on 1.5% agarose gels under UV illumination. Amplified PCR products were digested with selected restriction enzyme(s) specific for known recognition sites within the CYTB

fragment. Digestion reactions were performed according to the manufacturer's instructions, and fragments were separated on 2% agarose gels. Distinct banding patterns resulting from gain or loss of restriction sites were interpreted as SNP genotypes. Each fragment profile was scored as a presence (1) or absence (0) of bands to generate a binary matrix for analysis.

### 2.7 Data analysis

The binary SNP matrix was used to estimate genetic parameters, including:

- i. Allele frequencies
- ii. Number of segregating sites
- iii. Haplotype diversity (Hd)
- iv. Nucleotide diversity ( $\pi$ )
- v. Fixation index (Fst)
- vi. Gene flow (Nm)
- vii. Analysis of Molecular Variance (AMOVA)
- viii. Polymorphic relationships

All Analyses were performed using GenAlEx 6.5, Power Marker 3.25, MEGA 6.0 (Koichiro *et al.*, 2013), and the ETE3 Python package.

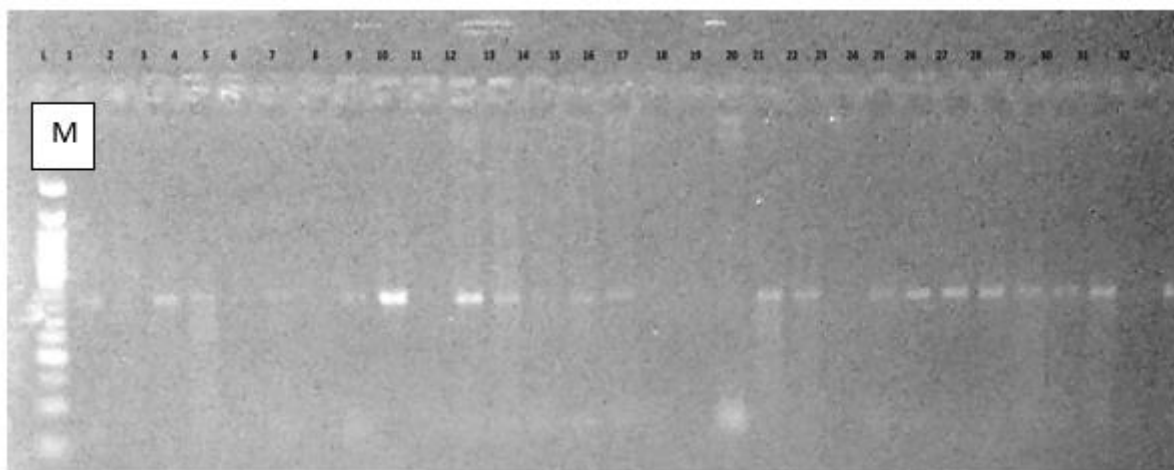
Phylogenetic trees were constructed to visualize genetic relationships, and bar charts were generated in Microsoft Excel 2016.

### 2.8 Population genetic structure and statistical analysis

Population differentiation between BS and WS ecotypes was quantified using Wright's fixation index (Fst), calculated from haplotype frequencies. Gene flow (Nm) was estimated indirectly using:  $Nm = \frac{1-Fst}{4Fst}$ , where Nm represents the number of migrants per generation. Analysis of Molecular Variance (AMOVA) was conducted to partition genetic variation among and within ecotypes. The significance of Fst and AMOVA variance components was tested using 999 permutations.

### 3.0 Results and Discussion

The results gel picture of cytochrome b bands for the black-skinned and white-skinned ecotype snails' samples are shown in plate 1 below. M is 100bp DNA ladder.



**Plate 1:** Gel picture of cytochrome B bands for the snail samples. M is 100bp DNA ladder

### 3.1 Genetic diversity within ecotypes

Genetic diversity indices for the black-skinned (BS) and white-skinned (WS) ecotypes are shown in Table 1 and figure 4 respectively. A total of 117 segregating sites were found for the black-skinned ecotype, while 185 segregating sites were found for the white-skinned ecotype. In addition, the number of haplotypes found for the black-skinned ecotype was 10, while the

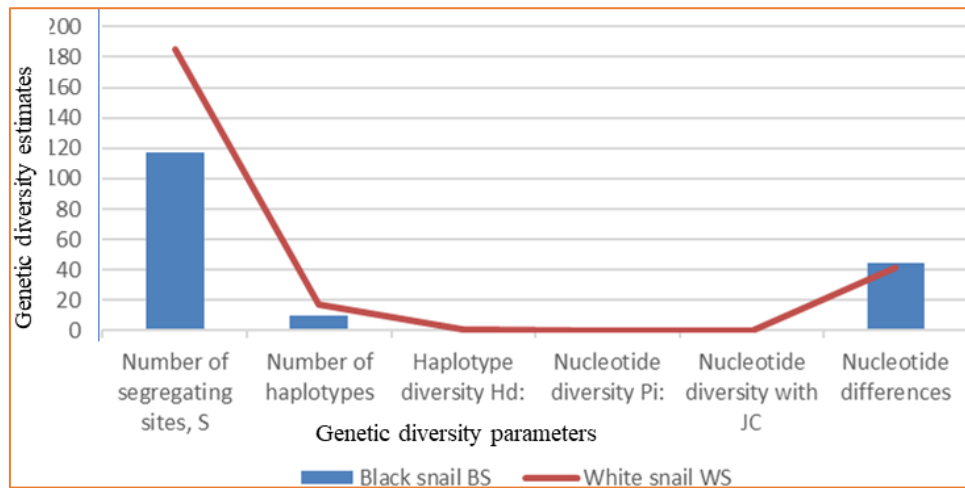
number found for the white-skinned ecotype was 17. The haplotype diversity, Hd, was found to be extremely high for the black-skinned and white-skinned ecotypes, at 1.000 and 0.9684, respectively. This suggests that the mitochondrial DNA pool for these ecotypes was diverse. In comparison, the nucleotide diversity,  $\pi$ , was slightly higher for the black-skinned ecotype (0.2093) compared to the white-skinned ecotype (0.1928). In the same

respect, the average number of nucleotide differences was slightly higher for the black-skinned ecotype (44.5778) compared to the

white-skinned ecotype (41.0632). This suggests that the black-skinned ecotype had slightly more genetic variation at the nucleotide level.

**Table 1:** Genetic diversity indices of black-skinned and white-skinned ecotypes

Parameters	Black snail (BS)	White snail (WS)
Number of segregating sites (S)	117	185
Number of haplotypes	10	17
Haplotype diversity (Hd)	1.0000	0.9684
Nucleotide diversity ( $\pi$ )	0.2093	0.1928
Nucleotide diversity (JC)	0.2527	0.2636
Nucleotide differences	44.5778	41.0632



**Figure 4:** Chart tie showing genetic diversity indices of black-skinned and white-skinned Ecotypes

These findings differ from those of Liew *et al.* (2010), who conducted a study on the genetic differentiation between the black-skinned and white-skinned ecotypes of giant African land snails and found that the haplotype diversity was found to be higher in the white-skinned ecotype than in the black-skinned ecotype. Inemotimineri *et al.* (2023) found that the nucleotide diversity was found to be higher in the white-skinned ecotype than in the black-skinned ecotype. Also, a study by Etukudo *et al.* (2016) on the genetic differentiation between the black-skinned and white-skinned ecotypes of giant African land snails in Calabar, Nigeria found that the average number of differences was found to be higher in the white-skinned ecotype than in the black-skinned ecotype. This differences nucleotide

diversity between black-skinned and white-skinned ecotype may be due to different genes or loci, or mutation rate variation.

### 3.2 Population genetic structure and differentiation

#### 3.2.1 Fixation Index (Fst) and Gene Flow (Nm)

The level of genetic differentiation between the black-skinned (BS) and white-skinned (WS) ecotypes was quantified using Wright's fixation index, Fst. The overall Fst was 0.008, revealing an extremely low level of genetic differentiation between the two black-skinned and white-skinned ecotypes snails.

Normally, Fst values that are less than 0.05, according to Wright's classification (Wright,

1949), reveal negligible levels of genetic differentiation. Therefore, the results obtained in the present investigation reveal that the mitochondrial genome has not been differentiated on the basis of the two colour morphs.

The level of gene flow (31 migrants per generation), was found to be present between the ecotypes, as inferred from the  $F_{st}$  values. A level of gene flow greater than 1 ensures that the effects of genetic drift are overcome, and values greater than 10 ensure that the effects of genetic drift are almost completely overcome. Therefore, the results obtained in the present

investigation strongly support the inference that there was no reproductive isolation between the two colour morphs.

The results obtained are consistent with the mixed pattern of clustering between the black-skinned and white-skinned ecotypes, as revealed from the phylogenetic trees (Figures 4 and 5).

### 3.22 Analysis of Molecular Variance (AMOVA)

The AMOVA results are presented in Table 1. The variation among the ecotypes was not statistically significant, as confirmed by the permutation test ( $P = 0.312$ ,  $P > 0.05$ ).

**Table 2:** AMOVA for Black- and White-Skinned Ecotypes

Source of Variation	Df	Sum of Squares	Variance Components	Percentage of Variation (%)	P-value
Among ecotype	1	0.012	0.001	0.8	0.312
Within ecotype	46	1.485	0.120	99.2	-
Total	47	1.497	0.121	100	-

Analysis of Molecular Variance (AMOVA) (Table 2), was used to apportion total mitochondrial genetic variation into components that correspond to variation among ecotypes and within ecotypes. AMOVA results showed that 0.8% of total genetic variation was among ecotypes, while 99.2% of total genetic variation was within ecotypes. Permutation tests (999 permutations) showed that the variance component among ecotypes was not statistically significant ( $P > 0.05$ ). The large percentage of variation within ecotypes supports the idea that the two-color morphs share a common maternal gene pool. While considerable divergence was found in certain black-skinned lineages (CYT\_12 and CYT\_15), this did not translate to overall genetic structure.

### 3.3 Estimation of genetic distance/diversity within and between white and black skinned ecotype snails

The genetic distance and diversity within and between white and black skinned giant African

land snail (*A. marginata*) are shown in Table 3. The mean genetic distance within the white skinned (WS) was 0.5362, while that of Black skinned (BS) was 0.3555. The pairwise mitochondrial genetic distance matrix showed a large amount of variation among the haplotypes. White-skinned individuals grouped together as a tightly knit group with small genetic distances (0.04-0.10), indicating recent common ancestry and high maternal gene flow. However, some black-skinned individuals showed small to moderate genetic distances with the white-skinned individuals, indicating the sharing of common mitochondrial haplotypes and the lack of strict genetic differentiation on the basis of skin color. On the other hand, some black haplotypes, especially CYT\_12 and CYT\_15, showed large genetic distances (0.45-0.63), indicating the presence of deeply diverged maternal lineages in the population.

The values obtained, 0.3555 for black skinned and 0.5362 for white skinned, may indicate a

slightly larger population of WS ecotype, thus showing diversity when compared to the black skinned ecotype in the Niger Delta region. This may also be in agreement with the findings of Okon *et al.* (2013). According to Uboh *et al.* (2010), there is cultural discrimination against the consumption of white skinned snails in most parts of the Cross River and Niger Delta region. This may mean that more black-skinned than white skinned ecotype is removed from the wild, thus showing that there is a slightly larger population of white skinned ecotype in the region. The mean genetic distance that exists between the white skinned and black skinned ecotype of *Archachatina marginata*, as shown in Table 3, is 0.470. This result is similar to that found by Okon *et al.* (2013), who found that the genetic distance was ranging from 0.370 to 0.40. This implies that the genetic difference that exists between the two-snail ecotype is low. This result agrees with the findings of Afolayan and Awodiran. (2015), who found that low genetic differences or variability that exist among *Archachatina marginata* snails may be attributed to the fact that the population of these snails in the site of study may be relatively small, which may have resulted in a high level of inbreeding. It may also be noted that the mean genetic distance that exists within the white ecotype, 0.5362, is greater than that found between the two ecotypes, 0.470. This may be attributed to the fact that there has been a selection process that has resulted in the high degree of homogeneity that exists among the black ecotype, since this ecotype is the most consumed among the two ecotypes, as found by Uboh *et al.* (2010).

The phylogenetic tree (Fig. 5) had formed into three clusters. Cluster I had a mix of black and white-skinned haplotypes, showing common maternal origins. Cluster II had predominantly white-skinned haplotypes, showing low genetic divergence as inferred by the low length of the branches. Cluster III had predominantly black-skinned haplotypes, showing considerable

divergence of the mitochondrial genomes. Intermixing of the two colours in these clusters indicates that there was no segregation of the mitochondrial genomes based on the skin colours. From the phylogenetic tree, it was evident that there was partial mitochondrial structuring of the different colour morphs. White-skinned haplotypes were grouped into a tight cluster with short branch lengths, showing that these haplotypes were not highly divergent. On the other hand, black-skinned haplotypes were highly heterogeneous, with some of them showing high divergence, especially CYT\_12 and CYT\_15. There is evidence of mixed clustering of black and white haplotypes, which shows that there is no segregation of mitochondria based on skin colour.

The circular phylogenetic tree (Fig. 6) was resolved into three clusters. Cluster I included a combination of black- and white-skinned haplotypes. This shows the common maternal ancestry of the haplotypes. Cluster II was mainly composed of white-skinned haplotypes with short branch lengths. These characteristics show low genetic divergence and a common ancestry. Cluster III included mainly black-skinned haplotypes such as CYT\_12 and CYT\_15 with long branch lengths, indicating a high level of mitochondrial divergence. The presence of mixed clusters shows the absence of mitochondrial segregation in the haplotypes.

### 3.2 Mitochondrial DNA polymorphism

Table 4 and Fig. 7, indicates the Mitochondrial DNA polymorphism in the black and white skinned ecotypes of the African giant land snails. The average number of sites for the black skinned ecotype is 292.31, for the white skinned ecotype is 318.02, and the combined average for both is 304.78.

**Table 3:** Mean group distance within white-skinned and black-skinned ectotype snails (*Archachatina marginata*)

CYT_42_{white}	CYT_39_{black}	CYT_38_{white}	CYT_36_{white}	CYT_35_{white}	CYT_29_{black}	CYT_27_{white}	CYT_26_{black}	CYT_25_{white}	CYT_23_{black}	CYT_22_{black}	CYT_20_{white}	CYT_19_{black}	CYT_15_{black}	CYT_14_{black}	CYT_12_{black}	CYT_11_{black}	CYT_9_{white}	CYT_4_{white}	CYT_3_{white}
CYT_42_{white}																			
CYT_39_{black}	2.7892																		
CYT_38_{white}	2.2518	0.0995																	
CYT_36_{white}	2.0791	0.0860	0.0511																
CYT_35_{white}	2.1827	0.0970	0.0917	0.0929															
CYT_29_{black}	2.1972	0.1388	0.0657	0.0727	0.1196														
CYT_27_{white}	2.5004	0.0753	0.0645	0.0584	0.0924	0.1048													
CYT_26_{black}	2.6113	0.1555	0.1195	0.1320	0.1502	0.1224	0.1171												
CYT_25_{white}	2.4049	0.1897	0.1622	0.1487	0.1792	0.1743	0.1599	0.1346											
CYT_23_{black}	2.0169	0.1084	0.0786	0.0760	0.0965	0.0982	0.0897	0.1108	0.1501										
CYT_22_{black}	2.3980	0.1379	0.1359	0.1333	0.1299	0.1316	0.1145	0.1135	0.1622	0.1126									
CYT_20_{white}	2.4978	0.0932	0.0890	0.0793	0.0929	0.0981	0.0862	0.1138	0.1706	0.0869	0.1326								
CYT_19_{black}	2.4494	0.2670	0.2537	0.2449	0.2802	0.2543	0.2446	0.2435	0.2936	0.2563	0.2738	0.2429							
CYT_15_{black}	2.5881	0.4825	0.4168	0.3976	0.4655	0.4028	0.3942	0.3677	0.3564	0.4077	0.3641	0.4263	0.5446						
CYT_14_{black}	2.0781	0.4580	0.4238	0.4003	0.4179	0.3881	0.4273	0.3911	0.3897	0.4246	0.3603	0.4117	0.4638	0.3721					
CYT_12_{black}	1.9986	0.5637	0.5607	0.5553	0.5605	0.5474	0.5639	0.5295	0.5177	0.5566	0.5411	0.5509	0.6291	0.4612	0.5348				
CYT_11_{black}	2.5967	0.4170	0.4001	0.3925	0.4083	0.3833	0.3959	0.3891	0.3599	0.3967	0.3913	0.4149	0.4460	0.3267	0.3054	0.6277			
CYT_9_{white}	2.3602	0.0684	0.0381	0.0482	0.0857	0.0909	0.0581	0.1246	0.1524	0.0757	0.1148	0.0793	0.2361	0.4180	0.4104	0.5672	0.4099		
CYT_4_{white}	2.2199	0.0684	0.0381	0.0482	0.0857	0.0909	0.0581	0.1246	0.1524	0.0757	0.1148	0.0793	0.2361	0.4180	0.4104	0.5672	0.4099	0.0680	
CYT_3_{white}	2.3898	0.0511	0.0510	0.0447	0.0890	0.0933	0.0413	0.1206	0.1634	0.0861	0.1368	0.0859	0.2559	0.4134	0.4497	0.5607	0.4149	0.0442	0.0415

The average number of differences for the black skinned ecotype is 77.422, for the white skinned ecotype is 65.689, and the combined average for both is 73.774. The number of polymorphic sites for the black skinned ecotype is 210, for the white skinned ecotype is 247, and the combined for both is 292. The differences in these figures between the black and white skinned snails could be the result of the effects of various evolutionary forces

that have acted in the populations over time (Gonzalez, 2020). In the natural world, a polymorphism is the term used to refer to the presence of genetic variation when the phenotypic variation in populations of the same species is maintained and caused by two or more alleles that have considerable recurrence (Mckinnon & Pierotti, 2010).

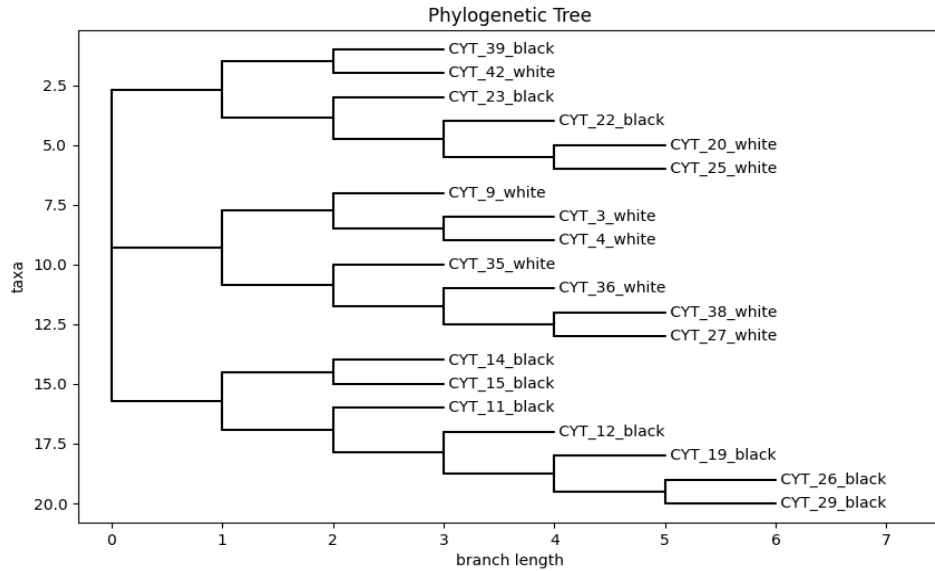


Figure 5: Phylogenetic tree of the black-skinned and white-skinned ecotype snails

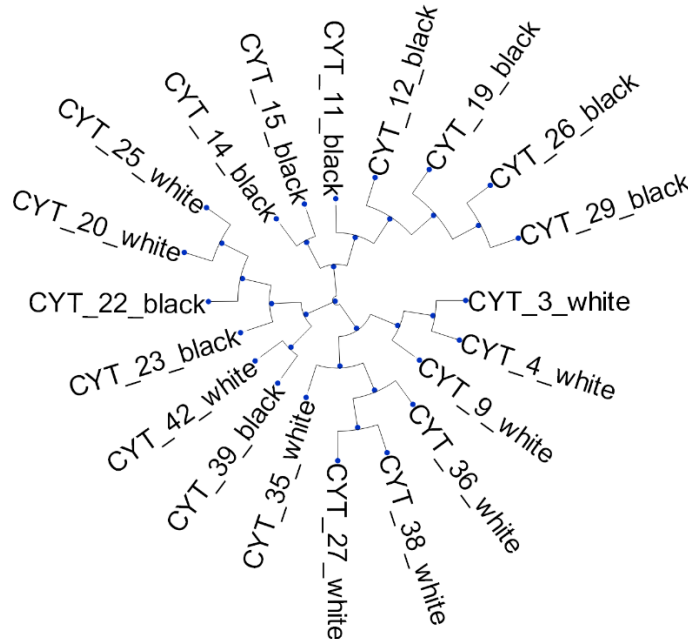
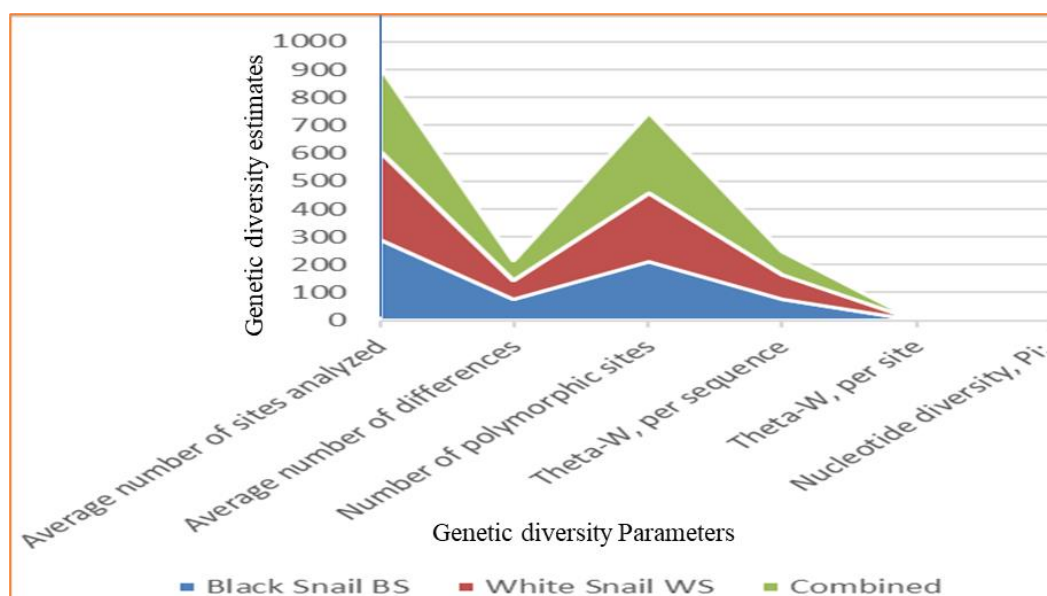


Figure 6: Circular phylogenetic tree of the black-skinned and white-skinned ecotype snails with scale bar representing genetic distance

**Table 4:** Mitochondrial DNA polymorphism

Parameters	Black Snail BS	White Snail WS	Combined
Average number of sites analyzed	292.31	318.02	304.78
Average number of differences	77.422	65.689	73.774
Number of polymorphic sites	210	247	292
Theta-W, per sequence	77.193	87.924	83.602
Theta-W, per site	0.2346	0.2673	0.2541
Nucleotide diversity, Pi:	0.2716	0.2104	0.2552

**Figure 7:** Chart showing Mitochondrial DNA polymorphism of black-skinned and white-skinned Snails

The weighted mean effect (Theta-W) per sequence is 77.193 for black colour, 87.924 for white colour, and 83.602 for the combination of both colours. The weighted mean effect (Theta-W) per site is 0.2346 for black colour, 0.2673 for white colour, and 0.2541 for the combination of both colours. Variation in the above parameters in snails on the basis of colour was also reported by Gonzalez, (2020). Variation in colour in the animal kingdom is important in science for the determination of the principles of biology, especially in genetics and evolution (Gonzalez, 2020). Nucleotide diversity

for black colour is 0.2716, for white colour is 0.2104, and 0.2552 for the combination of both colours. The values of nucleotide diversity are low and similar. Gonzalez (2020) has attributed this variation to random genetic drift, gene flow, natural selection, and mutation.

#### 4.0 Conclusion

This study shows that colour polymorphism is linked with partial mitochondrial genetic structuring in *Archachatina marginata* snails. However, it does not support the presence of genetic differentiation between black-skinned and

white-skinned ecotypes. White-skinned ecotypes showed a tight group with low genetic divergence, while black-skinned ecotypes showed a higher level of genetic heterogeneity with highly divergent maternal lineages. High haplotype diversity was found among both ecotypes, indicating a large amount of genetic variation. Additionally, the intermixing of haplotypes among the phylogenetic clusters indicates the presence of gene flow between the ecotypes. This shows that the ecotypes do not exhibit genetic isolation by skin colour. However, the low sample size and the use of only mitochondrial SNP markers were the main limitations of the study. Future studies need to include a large and geographically diverse sample with other molecular tools or the entire genome to fully elucidate the genetic basis and evolutionary significance of colour polymorphism in the giant African land snails.

### Acknowledgement

This research was supported by Topfaith University, Mkpatak, Nigeria, through the 2024 Research grant Cycle (Grant No.: 2024RG-TU320230059).

### References

- Afolayan, C. O. & Awodiran, M. O. (2015). Genetic differentiation of *A. marginata* populations from three vegetation zones using Random Amplified Polymorphic DNA Polymerase Chain Reaction. *Notulae Scientia Biologicae*. 7(3):372-381.
- Etukudo, O. M., Okon, B., Ekaluo, U. B. & Ibom, L. A. (2016). Genetic differentiation between black-skinned and white-skinned ecotypes of giant African land snails (*A. marginata*) in Calabar, Nigeria. *Global Journal of Pure and Applied Sciences*. 22: 135-139.
- Etukudo, O. M. (2017). Genetic diversity of giant African land snails using Inter Simple Sequence Repeat (ISSR) markers. *Ph.D. Thesis*, Department of Genetics and Biotechnology, University of Calabar, Calabar, Nigeria. Pp.120.
- Etukudo, O. M., Asuquo, B. O., Ekaluo, U. B., Okon, B. Ekerette, E. E., Umoyen, A. I., Udensi, O. U., Ibom, L. A., Afiukwa, A. C. & Igwe, D. O. (2018). Evaluation of Genetic Diversity in Giant African Land Snails Using Inter Simple Sequence Repeats (ISSR) Markers. *Asian Journal of Advances in Agricultural Research*. 7(2): 1-13.
- Etukudo, O. M., & Okon, B. (2025). Phenotypic landscape of giant African land snails. *Computing and Applied Sciences Impact*. 2(1): 1–12.
- Eze, J. N., Daisy, E., Akpodiete, O. J. & Okonkwo, J. C. (2010). Feeding Pattern, Carcass and Shell qualities of Snails (*Archachatina marginata*) Fed Different Material. *Journal of Innovative Research in Engineering and Science*. 1 (1): 111 – 121.
- Galeotti, P., Rubolini, D., Dunn, P. O., & Fasola, M. (2003). Colour polymorphism in birds; causes and functions. *Journal of Evolutionary Biology*. 16(4): 635-646.
- Gonzalez, D. R. (2020). Colour polymorphism in the terrestrial snail *Cepaea nemoralis*: from genetics and genomics to spectroscopy and deep learning. *A PhD Thesis* submitted to the University of Nottingham, Pp. 256.
- Gupta, N. (2019). DNA extraction and Polymerase Chain Reaction. *Journal of Cytology*. 36(2): 116-117.
- Ibom, L. A., Patani, I., Okon, B., & Okon, F. I. (2016). Phenotypic Variations in reproductive

- traits of two strains of *Archachatina marginata* snails (VAR. Ovum and Var. Saturalis) and their crosses. *Proceeding of the 5th annual international conference/workshop on Giant African land snails NetGals*. June 5-9, 2016. Pp. 36 – 42.
- Inemotimineri, P., Ogundu, U. E., Okoro, V. M. O. & Kadurumba, O. E. (2023). Evaluation of Mutation Type Associated with SNPs of Black Skinned and White Skinned Snails (*Archachatina marginata*) in the Rainforest Zone of Nigeria. *International Journal of Innovative Science and Research Technology*. 8(12): 1013-1018.
- Inemotimineri, P., Etukudo, O. M., Dudutari, P. E. & Kingsley, D. D. (2025). Reproductive Traits Variability in Black-Skinned and White-Skinned *Archachatina marginata*. *Acta Scientific Veterinary Sciences*. 7(8): 68-75.
- Koichiro, T., Glen, S., Daniel, P., Alan, F. & Sudhir, K. (2013). MEGA6: Molecular evolutionary Genetics analysis version 6.0. *Molecular Biology and Evolution*. 30:2725-2729.
- Liew, T. S., Schilthuizen, M. & Lakim, M. (2010). The determinants of land snail diversity along a tropical elevational gradient: insularity, geometry and niches. *Journal of Biogeography*. 37: 1071–1078.
- Odeh, M. O., Aikpokpodion, P. O. & Brisibe, E. A. (2015). Molecular characterization of giant African land snails using PCR-RAPD DNA fingerprinting. *Animal Molecular Breeding*, 5(1):1-8.
- Okon, B. & Ibom, L. A. (2012). *Snails breeding and snailery management*. Calabar: Freshdew Publication. p.90.
- Okon, B., Ibom, L. A., Williams, M. E. & Ekong, N. B. (2012). Effects of breed on reproductive Efficiency of two most popular snails (*A. marginata* (S) and *A. achatina*(L)) in Nigeria. *Journal of Agricultural Science*. 4(8):236-245.
- Okon B., Ibom, L. A., & Njume, G. N. (2013). Genetic differentiation between the black-skinned and white-skinned snails (*Archachatina marginata*) using random amplified polymorphic DNAs. *African Journal of Biotechnology*. 12(12), 5035-5039.
- Mckinnon, J. S., & Pierotti, M. E. R. (2010). Colour polymorphism and correlated characters: genetic mechanisms and evolution. *Molecular Ecology*. 19:5101-5125.
- Uboh, F. E., Ebong, P. E., & Mbi, E. (2010). Cultural discrimination in the consumption of black snail (*A. marginata*) and white snail (*A. achatina*); Any scientific justification? *International Research Journal of Microbiology*. 1:13-17.
- Wright, S. (1949). The general structure of populations. *Ann Eugenics*. 15: 323-354.