

Chromosome numbers, evolutionary relationships and divergence among three breeds of giant african land snails in Nigeria

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Abstract

A number of studies have been carried out on the reproductive and growth performance of these breeds of giant African land snails, but not much is documented on chromosome, evolutionary relationships and divergence studies. Forty snails and 10 of each breed of giant African land snails *Archachatina marginata* (AM), *Achatina achatina* (AA) and *Achatina fulica* (AF) and two varieties of *A. marginata* [*A. marginata* var. *saturalis* (AMS) and *A. marginata* var. *ovum* (AMO)] were used for the chromosomes numbers analyses. Slides for chromosome identification were prepared using the ovotestes and the cells were examined for spread at metaphase. The haploid (*n*) chromosome numbers obtained revealed and confirmed that AF, AA, AMS and AMO snails have 27, 30, 28 and 28 chromosomes respectively. Also 13 amino acid sequences were retrieved from the National Centre for Biotechnology Information with accession numbers: ALD09273, AAY62497, ACA 10148 and AKQ 76237 for AM; AKQ 76253, AKQ 76250, CDL 67813, CDL 67813 and AKQ 76249 for AA and SP/P35903, PDB/5CZL, KZM 80032 and YP009049167 for AF snails. The evolutionary history was inferred using the Neighbour-Joining method. Phylogenetic tree analysis by Neighbour-Joining (NJ) was constructed using 10 out of the 13 amino acid sequences. The reliability of the tree was calculated by bootstrap confidence value with 1000 bootstrap iterations using MEGA 7 software. The phylogenetic trees showed that these snail breeds are highly divergent. *A. achatina* was separately related to itself; whereas *A. marginata* slightly mingled with *A. fulica*. The degrees of divergence obtained signified that they have evolved from different ancestors. The evolutionary distances were computed with the remaining three amino acid sequences using the Poisson Correction method. Very high and far apart average genetic distances of 0.923 ± 0.018 , 0.926 ± 0.018 and 0.926 ± 0.017 were obtained for AA vs AF, AF vs AM and AA vs AM snails respectively, confirming that these snail breeds are genetically far apart between themselves. The results obtained for chromosome numbers, evolutionary relationship and average genetic distances might be a panacea in further selection and molecular classification of giant African land snails (GALS) in Nigeria.

Keywords: Chromosome number, Evolutionary distance, Breed, snail, Nigeria

Introduction

Nigeria is greatly endowed with large number of land snails and its domestication and rearing is recently receiving more attention because of its positive impact on agricultural, industrial, pharmaceutical and economic transformation agenda (Okon,

2015); as well as in pharmaceutical/cosmetic industry in Nigeria (Akpa, 2015). According to Sam *et al.* (2012), snails are one of the commonest sources of animal protein in Southern Nigeria where the ecology favours their continued existence. The meat is high in protein (12 – 16%) and iron (45 –

Divergence among three breeds of giant african land snails in Nigeria

50mg/kg), low in fat (0.05 – 0.08%) and contains all the amino acids needed for human nutrition (NAERLS, 1995). They are also rich in vitamins A, E, K and B₁₂ as well as essential fatty acids. Also, giant land snails are great source of the valuable lectin which possesses anti-cancer properties that help in the boosting of immune system. The immune system, according to Onuorah (2017) is better equipped to fight against cancerous cells. Besides, the low cholesterol content of snails makes them suitable meat for those suffering from

diabetes, hypertension and other heart diseases (Onuorah, 2017). The giant African land snails (GALS) which belong to the family *Achatinidae*, are made up of four (4) breeds namely; *Archachatina marginata* (Plate 1), *Achatina achatina* (Plate 2), *Achatina fulica* (Plate 3) and *Archachatina degneri* (Plate 4). But Omole *et al.* (2000), Okon and Ibom (2012) and Okon (2013) opined that four different popular breeds of snail found and reared in Nigeria include *Archachatina marginata*, *Achatina achatina*, *Achatina fulica* and *Limicolaria* species.



Plate 1: *Archachatina marginata* snail



Plate 2: *Achatina achatina* snail



Plate 3: *Achatina fulica* snail



Plate 4: *Archachatina degneri* snail

Of these species, Smith and Fowler (2003) pointed out that only *Achatina achatina* and *Archachatina marginata* are “truly giant African land snails” as the largest specimens recorded by [PPQ – Plant Protection Quarantine] have been in the range of 750 g and 8 inches shell”. Whereas, Adikwu (2012) and Aluko *et al.* (2017) noted that the giant African land snails consist of only three species namely *Achatina achatina*, *Achatina fulica* and *Archachatina marginata*, probably because of the smaller size of the *Limicolaria* species. Chromosomes are composite structures with well-defined structures that play vital roles in the process of replication, transcription and regulation of gene expression. The chromosome number varies among the different breeds of snails, therefore crossbreeding principles cannot be applied between and among them for genetic improvement; that is they cannot breed true. Thus, Affonso *et al.* (2007) pointed out that the basic information of chromosome number and morphology coupled with simple breeding techniques and light microscopy analysis are useful to explain a variety of subjects ranging from cytotaxonomy to karyotype. Awodiran *et al.* (2012) reported the haploid (n) chromosome number of 28, 30, 27 and 24 for *Archachatina marginata*, *Achatina achatina*, *Achatina fulica* and *Archachatina papyracea* snails respectively. Whereas, Fagbuaro *et al.* (2002) and Idowu and Akinnusi (2006) had earlier observed the diploid (2n) chromosome number of 56, 44, 54, 28 and 56 for *Archachatina marginata*, *Achatina achatina*, *Achatina fulica*, *Limicolaria aurora* and Aquatic snails respectively. Phylogenetic analysis plays an important role in the investigation of species diversity as well as novel species identification (Surakasi *et al.*, (2007). Thus, Bibinu *et al.*

(2016) opined that the phylogenetic tree is used to compare the common ancestral nucleotide and amino acid sequences of the evolutionary relationships as well as for providing information on an evidence of trans-species evolution which might be attributed to the coding nature of the sequence. A lot of research studies have been done and reported on the reproductive and growth performance of these breeds of giant African land snails, but not much is documented on chromosome studies, evolutionary relationships and divergence. Therefore, this study seeks to determine the chromosome numbers of the two varieties of *Archachatina marginata* snails, three breeds of giant African land snails, evolutionary relationships and their divergence.

Materials and methods

Forty snails, 10 of each breed of mature snails *Archachatina marginata* (AM), *Achatina achatina* (AA), *Achatina fulica* (AF), and two varieties of *Archachatina marginata*; *A. marginata var. saturalis* (AMS) and *A. marginata var. ovum* (AMO) were used for the chromosome analysis at the Obafemi Awolowo University, Ile-Ife, Nigeria. The preparation of slides for the identification of chromosome numbers using the ovotestes were done as described by Bassey (2017). The slides were examined following the method of Fagbuaro *et al.* (2002). Cells that were adjudged to contain well spread metaphase chromosomes were eye karyotyped and then photographed. The photography was done under oil immersion using a photo microscope (PWBK5000T, PROWAY OPTICS, China). The eye-karyotyping method helped more accurately by identifying homologous chromosome pairs as well as differentiate and separate artifacts which could easily be confused with small chromosomes on the photographs/plates.

Divergence among three breeds of giant african land snails in Nigeria

A total of 13 amino acid sequences were retrieved from the National Centre for Biotechnology Information with accession numbers: ALD09273, AAY62497, ACA10148 and AKQ76237 for *Archachatina marginata*, AKQ76253, AKQ76250, CDL67813, CDL67813 and AKQ76249 for *Achatina achatina* and SP/P35903, PDB/5CZL, KZM80032 and YPO09049167 for *Achatina fulica* snail. The evolutionary history was inferred using the Neighboring-Joining method (Saitou and Nei, 1987). Ten amino acid sequences were re-selected from the above 13 sequences for the optimal tree with the sum of branch length equals 10.51798688 as shown; while the remaining three amino acid sequences were used for the estimation of evolutionary divergence. The percentage replicate trees in which the associated taxa clustered in the bootstrap test (1000 replicates) are shown next to the branches (Felsenstein, 1985). The phylogenetic tree

was drawn to scale, with the branch lengths in the same units as those for evolutionary distances. The evolutionary distances were computed using the Poisson correction method (Zuckerukandl and Pauling, 1965) and are in the units of the number of amino acid substitution per site. All positions containing gaps and missing data were eliminated. There were a total of 170 positions for the phylogenetic tree and 240 positions for the genetic distances in the final data set. The evolutionary analyses were conducted with MEGA 7 software (Kumar *et al.*, 2016).

Results and discussion

The number of cells spread examined, the modal haploid and diploid chromosome numbers obtained for *A. fulica*, *A. achatina* and *A. marginata* snails are shown in Table 1. The number of spreads used for the chromosome studies of the three breeds and two varieties were large and ranged between 180 and 205.

Table 1: The number of metaphase cells, the modal haploid and diploid chromosome numbers of *A. fulica*, *A. achatina*, *A. marginata* var. *Ovum* and *A. marginata* var. *Saturalis*

Breed/Variety	Number of spread	Modal haploid Number	Modal Diploid Number
<i>A. fulica</i>	205	27	54
<i>A. achatina</i>	200	30	60
<i>A. marginata</i> var. <i>Ovum</i>	180	28	56
<i>A. marginata</i> var. <i>Saturalis</i>	200	28	56

The representative spreads for each breed and variety are shown on Plate 5 (*A. fulica* chromosomes), Plate 6 (*A. achatina* chromosomes), Plate 7 (*A. marginata* var. *saturalis* chromosomes) and Plate 8 (*A. marginata* var. *ovum* chromosomes). The number of observed metaphase cells were

constant and consistently uniform in the morphology of chromosome structures studied, and is worthy of note in the study of chromosome numbers. Chromosomes of *A. marginata* (Plates 7 and 8) and *A. achatina* (Plate 6) snails were large and made up of metacentric and acrocentric types.

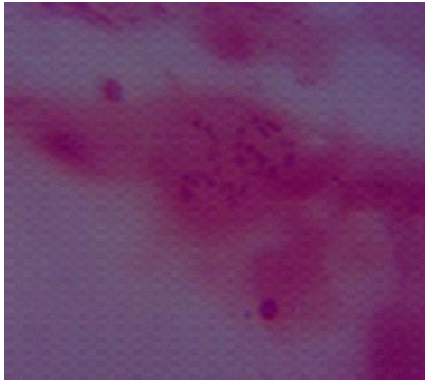


Plate 5: *A. fulica* chromosomes

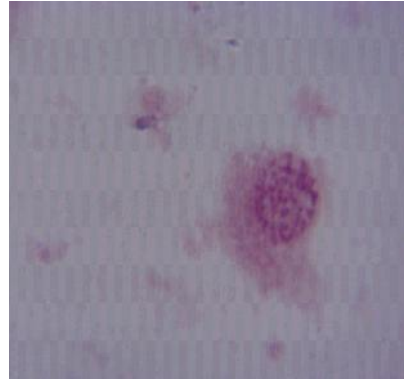


Plate 6: *A. achatina* chromosomes

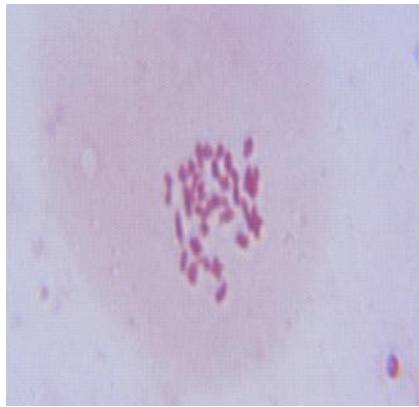


Plate 7: *A. marginata* var. *Saturalis* chromosomes

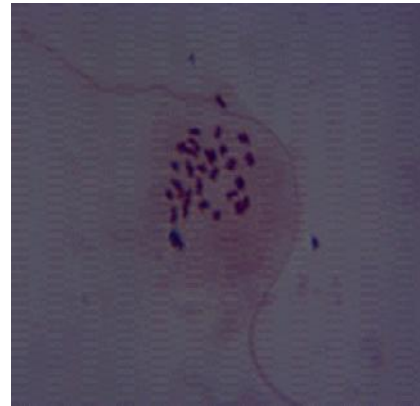


Plate 8: *A. marginata* var. *Ovum* chromosomes

Hence, supporting Awodiran *et al.* (2012) views that most of the chromosomes of *A. marginata* and *A. achatina* were large, maximally contracted and were made of metacentrics and acrocentrics while those of *A. fulica* were smaller and more of acrocentric types. The diploid (2n) chromosome numbers obtained for the snails studied were *A. fulica* (2n = 54), *A. achatina* (2n = 60), *A. marginata* var. *saturalis* (2n = 56) and *A. marginata* var. *ovum* (2n = 56) (Table 1). The haploid chromosome numbers obtained for *A. fulica* (n = 27) and *A. marginata* (n = 28) snails corresponded to and confirmed the same haploid chromosome numbers by Idowu and Akinnusi (2006), Okon and Ibom

(2012) and Aworidan *et al.* (2012). Whereas the haploid (n) chromosome number obtained in this study for *A. achatina* (n = 30) was quite higher than the n = 22 reported by Fagbuaro *et al.* (2002) and Idowu and Akinnusi (2006); but similar to Choudhury and Mohapatra (1991) and Aworidan *et al.* (2012) result of n = 30. The disparity observed in the haploid chromosome number might be attributed to variations in mitotic index and presence of large number of very small chromosomes (Choudhury *et al.*, 1992), as well as types and sizes of chromosomes observed/seen.

The Neighbour-Joining trees were constructed to compare the common ancestral nucleotide and amino acid

Divergence among three breeds of giant african land snails in Nigeria

sequences of the giant African land snails, since the trees may give useful information for proper understanding of the evolutionary relationships. The phylogenetic tree (Fig. 1) obtained revealed that giant African land snails are highly divergent among the breeds. *A. achatina* is related to itself because sequences from the same breed tend to cluster together than sequences from other breeds (Fig. 1). However, *A. marginata* slightly intermingled with *A. fulica*. The degree of divergence among these breeds signified that the breeds might have evolved from different ancestors (Dangre *et al.*, 2009). This is evidence of trans-species evolution which might be attributed to the coding nature of the sequence (Bibinu *et al.*, 2016). The assumption is that the gene tree, based on molecular data with all its advantages, is more accurate and less ambiguous representation of the species tree than that

obtained by morphological comparisons. Thus, Brown (2002) opined that this assumption is often correct. Such information emanating from this study would be relevant in further selection of giant African land snails especially for breeding to improve genetically giant land snails in Nigeria.

The results of estimates of evolutionary divergence between sequences of these snail breeds (*A. fulica*, *A. achatina* and *A. marginata*) are presented in Table 2. The upper diagonal represents standard error estimate(s); while the lower diagonal is the average genetic distance between breeds which is also known as the average nucleotide substitutions per site (Dxy). The Dxy values recorded for *A. fulica* versus (vs) *A. achatina* was 0.922 ± 0.018 , that of *A. fulica* vs *A. marginata* was 0.926 ± 0.018 and that of *A. achatina* vs *A. marginata* was 0.926 ± 0.017 (Table 2).

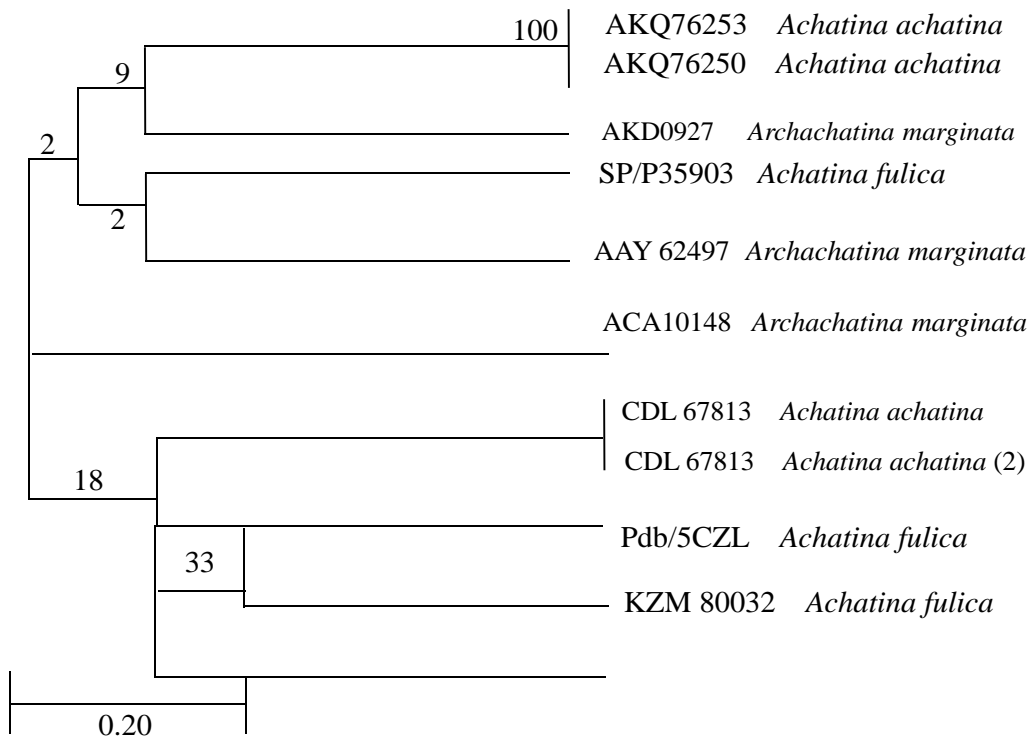


Fig. 1: Evolutionary relationships of giant African land snails

Table 2: Estimates of evolutionary divergence between sequences

Breed	<i>Achatina fulica</i>	<i>Achatina achatina</i>	<i>Archachatina marginata</i>
<i>Achatina fulica</i>	1.000	0.018	0.018
<i>Achatina achatina</i>	0.922	1.000	0.017
<i>Archachatina marginata</i>	0.926	0.926	1.000

These average genetic distances obtained in this study were very high. This means that these breeds of snails are genetically far apart between themselves, because the average genetic distance (Dxy) is an index of divergence between and among the breeds. Thus, Vincent *et al.* (2014) opined that the higher the value of Dxy, the far apart the breeds are, which by implication, higher values have lesser orthology and more paralog and vice versa. Similarly, Kang *et al.* (2008) noted that the larger the Dxy value, the greater the genetic distance; while the smaller the Dxy value, the closer the genetic distance between the breeds. On the other hand, Marini *et al.* (2010) observed ortholog divergence from common ancestors, their different evolutionary trajectories led to divergence in the selective constraints on homologous sites. The results of average genetic distances obtained here for these breeds of snails revealed that they might have evolved from different ancestors. Hence, these results may also be useful in further molecular classification.

Conclusion

The results of this study revealed and confirmed that the haploid (n) chromosome numbers of *A. fulica*, *A. achatina*, *A. marginata var. saturalis* and *A. marginata var. ovum* snails are 27, 30, 28 and 28 respectively. The phylogenetic tree showed that these breeds of snails are highly divergent. *A. achatina* was separately related to itself because sequences from the

same breed tend to cluster together than sequences from other breeds; whereas *A. marginata* slightly intermingled with *A. fulica*. The degrees of divergence among the snail breeds signified that they might have evolved from different ancestors. Besides, the average genetic distances obtained from the three breeds of giant African land snails are very high and far apart, confirming that the snail breeds are genetically far apart between themselves. The results obtained on chromosome numbers, evolutionary relationships and average genetic distances might be a panacea in further selection and classification of giant African land snails (GALS) in Nigeria.

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Divergence among three breeds of giant african land snails in Nigeria

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Divergence among three breeds of giant african land snails in Nigeria

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