Xi'an Shiyou Daxue Xuebao (Ziran Kexue Ban)/
Journal of Xi'an Shiyou University, Natural Sciences Edition
ISSN: 1673-064X
E-Publication: Online Open Access
Vol: 66 Issue 11 | 2023
DOI: 10.5281/zenodo.10224157

GENETIC DIVERSITY AND PHYLOGENETIC ANALYSIS OF NON-DESCRIPT AND HYLA RABBITS REARED IN NSUKKA, NIGERIA USING MITOCHONDRIAL DNA

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Abstract

Mitochondrial DNA (mtDNA) cytochrome b gene sequences were utilized to assess the genetic diversity and phylogenetic relationships of non-descript and Hyla rabbit populations in Nsukka, Nigeria. Non-descript rabbits, as locally adapted genotypes, play a crucial role in preserving genetic diversity and adaptability. Our analysis revealed both non-descript and Hyla rabbits as polymorphic, with non-descript rabbits exhibiting higher haplotype diversity (0.761±0.092) compared to Hyla (0.598±0.032). Nucleotide diversity ranged from 0.10137±0.00021 in non-descript to 0.06405±0.01057 in Hyla, underlining the genetic variation and adaptability of non-descript rabbits. The significantly negative Tajima's D value (-1.53783) in non-descript rabbits indicates natural selection and rare allelic combinations, further highlighting their unique genetic signature. Phylogenetic analysis unveiled two distinct clusters, with Hyla, New Zealand, and Californian rabbits forming one group and non-descript, Chinchilla, and Domestic English rabbits comprising the other. This suggests a history of genetic exchange and hybridization, particularly with Hyla possibly being a result of crossbreeding between Californian and New Zealand white rabbits. The genetic distinctiveness of non-descript rabbits reinforces their potential role in maintaining indigenous genetic resources. To harness the genetic potential of these rabbit populations, we recommend comprehensive genetic characterization incorporating nuclear DNA markers and quantitative traits. Breeding programs should prioritize the preservation and improvement of locally adapted genotypes, and efforts to conserve and utilize Animal Genetic Resources (AnGR) should focus on preserving unique genetic variants found in non-descript rabbits, thereby, addressing food security and promoting environmental sustainability in livestock production in Nigeria.

Index Terms: Non-Descript Rabbit, Hyla Rabbit, Mitochondrial DNA, Cytochrome B Gene, Genetic Diversity, Haplotypes, Polymorphs, And Phylogeny.

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INTRODUCTION

Nigeria, with its current population exceeding 219 million (Worldometerics, 2023), faces a critical challenge in meeting the increasing demand for livestock products. This challenge is exacerbated by factors such as insecurity, rising feed and medication costs (FAOSTAT, 2018), and ongoing issues like the conflict between grazers and farmers, religious taboos, and the soaring costs of feeding livestock (Omotoso *et al.*, 2019). As a consequence, the country's reliance on larger livestock, including ruminants, poultry, and pigs, for animal protein is gradually waning.

The dwindling numbers of these larger livestock, in part due to the turmoil in the North, highlight the need for alternative sources of animal protein to fulfill the dietary requirements of the Nigerian population. In light of this, several researchers have suggested the exploration of non-conventional livestock species, such as rabbits (Siddiqui *et al.*, 2023; Oosting *et al.*, 2022; Kumar *et al.*, 2022), to bridge the protein deficit.

Rabbits (Oryctolagus cuniculus) present themselves as a viable option for meat production in Nigeria, offering several advantages over other livestock species. They are known for their prolificacy, short gestation periods, low initial capital requirements, and adaptability to various environmental conditions. The reproductive efficiency of rabbits, marked by short gestation lengths and large litters, positions them as a species capable of significantly contributing to the availability of affordable and high-quality animal protein.

Rabbit meat is particularly attractive as it boasts high biological value protein, low sodium, low cholesterol, high unsaturated lipids (comprising 60% of total fatty acids), and is low in calories and fat (Siddiqui *et al.*, 2023). Despite these favorable traits, commercial rabbit rearing in Nigeria remains underdeveloped. Nigerian rabbit farmers grapple with challenges such as heat stress, limited access to feed and markets, indiscriminate mating practices, record-keeping deficiencies, and a lack of breed classification (Adeolu *et al.*, 2021).

Furthermore, the rapid replacement of local breeds with exotic ones has raised concerns about the potential loss of indigenous animal species. The Food and Agriculture Organization of the United Nations (FAO) has recognized this threat and encouraged conservation measures to prevent the irreversible decline of native animal species (FAO, 2019). The conservation and sustainable utilization of Animal Genetic Resources (AnGR) have gained importance in ensuring food security and environmental sustainability (Gowane *et al.*, 2019).

Efforts by animal breeders to establish breeding programs and gene banks for locally adapted breeds aim to provide diverse and resilient animal genetic resources for future generations (Eusebi *et al.*, 2019; Bolatito and Aladele, 2019). Nevertheless, there is a lack of information regarding the population history and genetic diversity of rabbits retained in Nigeria. The absence of this vital information is attributed to factors such as indiscriminate mating and the absence of records. As a result, it becomes imperative to determine genetic variation parameters in the existing rabbit populations and breeds.

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This knowledge is essential for developing long-term breeding strategies, formulating effective conservation policies, and providing valuable genetic data to enhance the improvement of this important genetic stock. The focus of this study, therefore, is to assess the genetic diversity and evolutionary relationships between the locally adapted non-descript and the Hyla rabbits reared in Nsukka, Nigeria, contributing to the broader context of conservation and sustainable utilization of animal genetic resources.

MATERIALS ANS METHODS

Study Location

The research was conducted at the Rabbit Unit of the Animal Science Research Farm, University of Nigeria, Nsukka, situated in Enugu State, South Eastern Nigeria. This location falls within the savannah region and is geographically positioned at approximately 6°51'3.308"N latitude and 7°25'36.48"E longitude, with an altitude of 443 meters above sea level. The climate is characterized as humid tropical, featuring daily minimum and maximum temperatures ranging from 33 to 37 degrees Celsius and an annual rainfall between 1680 and 1700 millimeters (Onyenucheya and Nnamchi, 2018).

Sample Collection and Population

The study population comprised 64 female and 16 male rabbits, representing both non-descript (ND) and Hyla (H) breeds. For each breed, there were 32 female and 8 male rabbits, sourced from different localities within Nsukka (Fig 1). The objective of this diverse selection was to ensure a high level of genetic variation within the study region.

DNA Analysis and Sequencing

Blood samples were collected from the assembled rabbit population. Genomic DNA was extracted using the GenaAid DNA extraction kit through a series of steps:

RBC Lysis Transfer: 900 µl of RBC Lysis Buffer and 300 µl of whole blood were mixed in a 1.5 ml microcentrifuge tube. The mixture was incubated for 5 minutes at room temperature and then centrifuged at 3,000 x g for 5 minutes to obtain a leukocyte pellet. The supernatant was carefully removed, leaving approximately 50 µl of residual buffer and leukocyte pellet. The tube was vortexed until the leukocyte pellet was fully resuspended.

Lysis: 300 µl of Cell Lysis Buffer was added to the tube, mixed by vortexing, and incubated at 60ºC for 10 minutes to ensure a clear and homogenous sample lysate. The tube was inverted every 3 minutes during incubation.

Protein Removal: 100 µl of Protein Removal Buffer was added to the sample lysate, vortexed for 10 seconds, and then centrifuged at 14-16,000 x g for 3 minutes to form a dark brown protein pellet.

DNA Precipitation*:* The supernatant was transferred to a clean 1.5 ml microcentrifuge tube, and 300 µl of isopropanol was added and mixed well. After centrifugation at 14-

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16,000 x g for 5 minutes, the supernatant was discarded, and 300 µl of 70% ethanol was used to wash the pellet. Following another centrifugation at 14-16,000 x g for 3 minutes, the supernatant was discarded, and the pellet was air-dried for 10 minutes.

DNA Rehydration: To dissolve the DNA pellet, 100 µl of DNA Hydration Buffer was added. The tube was gently vortexed for 10 seconds and incubated at 60ºC for 5-10 minutes, with occasional tapping to promote DNA rehydration.

DNA Concentration and Purity Assessment

The concentration and purity of the extracted DNA were determined using a NanoDrop 1000 Spectrophotometer. Additionally, potential DNA degradation was visualized on a 1% agarose gel.

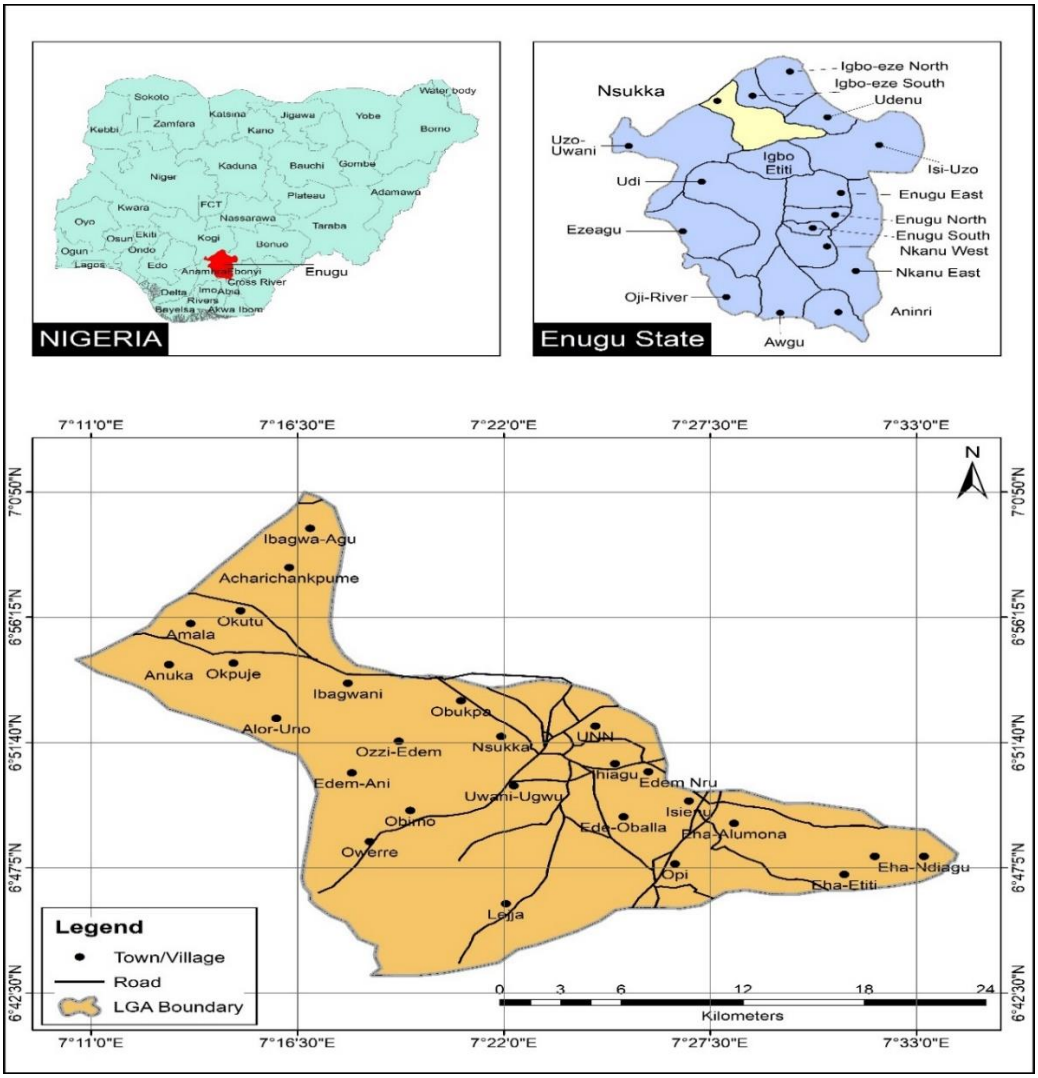

Fig 1: Map of Nsukka Local Government Area, Enugu State, Nigeria

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Mitochondrial DNA (mtDNA) Analysis

The mtDNA cytochrome b sequence (450 bp) was used in this study for further analysis. Polymerase chain reaction (PCR) amplifications were conducted using specific forward (Pro1) and reverse (NC4) primers. The nucleotide positions were numbered according to the complete published sequence of mtDNA (GenBank accession number AJ001588). The PCR mixture contained 1.0 µL of genomic DNA in a 9 µL mixture, which included 5 µL of Master Mix (Qiagen206145, Germany), 0.4 µL Pro1, 0.4 µL NC4 primers (Invitrogen, France), and 3.2 µL distilled water. PCR products were purified using EXOSAP-IT and Sephadex™. Sequencing was carried out with the BigDye® Terminator v3.1 Kit and analyzed on a DNA sequencer (ABI PRISM 3130 XL–Genetic Analyzer).

Statistical Analysis

To investigate the possible origin of the non-descript and Hyla rabbits and their relationships, reference rabbit mtDNA sequences (Table 1) were obtained from the National Centre for Biotechnology Information (NCBI). Sequence alignment was performed using DNASTAR software (DNASTAR Inc., Madison, WI, USA). Validation of breed purity was based on sequence alignment. Parameters such as the number of nucleotide polymorphic sites (S), haplotype (h), nucleotide diversity (π), haplotype diversity (Hd), and Tajima's D (TD) were calculated using DNA sequence polymorphism Version 5.1 (Librado and Rozas, 2009). Genetic distance among tested breeds was estimated using Mega 6.0 (Tamura *et al.*, 2013) with the unweighted pair group method with arithmetic mean (UPGMA).

Table 1: Sequence data list from Gene bank used in this study

Accession Number	Breed	Reference
AJ293835	Domestic English	Bolet *et al.* (2000)
AJ293842	Chinchilla	Bolet *et al.* (2000)
AF534086	California	Long *et al.* (2003)
AF534089	New Zealand	Long *et al.* (2003)

RESULTS AND DISCUSSION

Mitochondrial DNA (mtDNA) Diversity Indices: The examination of mtDNA diversity in this study provides valuable insights into the genetic makeup of non-descript and Hyla rabbit populations in Nsukka, Nigeria (Table 2). A total of six haplotypes were observed, highlighting the genetic diversity within these populations. Both non-descript and Hyla rabbits displayed polymorphism, with the number of haplotypes ranging from two in Hyla to six in non-descript rabbits.

Notably, non-descript rabbits exhibited a higher haplotype diversity (0.761±0.092) compared to Hyla rabbits (0.598±0.032). This discrepancy in haplotype diversity underscores the genetic variability present in non-descript rabbits, further supporting their status as a locally adapted genotype. These findings align with previous research

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demonstrating that larger and more heterogeneous populations tend to exhibit higher levels of genetic diversity (Sakthivel *et al.*, 2019).

Nucleotide diversity, an important measure of genetic variation within breeds, ranged from 0.06405±0.01057 (Hyla) to 0.10137±0.00021 (non-descript). This variation in nucleotide diversity signifies differences in the genetic makeup and adaptability of these rabbit populations. The higher nucleotide diversity observed in non-descript rabbits suggests a wider genetic pool and potential for adaptation to various environmental conditions, which is often associated with heterogeneous populations (Schumer *et al.*, 2018).

In contrast, Hyla rabbits displayed lower nucleotide diversity, possibly due to their more stable and homogeneous breed characteristics (Table 3). Stable breeds, like Hyla, may be more susceptible to bottleneck effects and inbreeding, which can lead to reduced genetic diversity (Bortoluzzi *et al.*, 2019). These results highlight the genetic distinctiveness between non-descript and Hyla rabbits and underline the importance of preserving the genetic diversity present in locally adapted populations.

Table 2: The mtDNA diversity indices of the tested rabbit populations

Genotype	No of Haplotypes	Nucleotide diversity±SD	Haplotype diversity±SD	No of polymorphic sites	Tajima's D (*P<0.05)
Non-descript	6	0.10137±0.00021	0.761±0.092	7	-1.53783*
Hyla	2	0.06405±0.01057	0.598±0.032	2	1.24253

Table 3: Mitochondrial DNA Haplotype Frequencies and lineage assignment

Haplotype	Non-descript	Hyla	Lineage
1	0.04	-	A
2	0.127	-	A
3	0.163	-	A
4	0.405	-	A
5	0.096	0.688	A
6	0.169	0.312	A

Tajima's D values, a measure of the departure from neutral evolution, were significantly negative (-1.53783) for the non-descript rabbits, indicating an excess of rare allelic combinations. This suggests that non-descript rabbits have undergone natural selection processes, leading to the accumulation of rare alleles. In contrast, there was no significant difference among Hyla rabbits, as their Tajima's D values did not deviate significantly from neutrality. These findings further emphasize the genetic uniqueness of non-descript rabbits, possibly resulting from their adaptation to local conditions (Guo *et al.*, 2019).

Phylogenetic Analysis: The phylogenetic analysis of tested rabbit breeds and published sequences revealed interesting insights into their evolutionary relationships (Fig 2). The UPGMA tree demonstrated that all tested groups fell into the same lineage (Table 3), highlighting a shared ancestry among these rabbit populations. However, the UPGMA tree also showed the presence of two distinct clusters. One cluster included Hyla rabbits, New Zealand rabbits, and Californian rabbits, suggesting a closer genetic relationship

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among these breeds. The formation of this cluster indicates a potential history of crossbreeding and genetic exchange between these breeds, with Hyla possibly being a product of hybridization between Californian and New Zealand white rabbits. In contrast, the other cluster comprised non-descript rabbits, Chinchilla rabbits, and Domestic English rabbits. This separation suggests that non-descript rabbits have a unique genetic lineage, distinct from the other breeds tested. This differentiation is in line with the observed high genetic diversity in non-descript rabbits, driven by indiscriminate mating practices and genetic recombination (Park *et al.*, 2019).

The results of this phylogenetic analysis provide crucial information about the genetic relationships and evolutionary history of these rabbit breeds. It also highlights the potential for genetic improvement and conservation strategies to preserve unique genetic variants within non-descript rabbits.

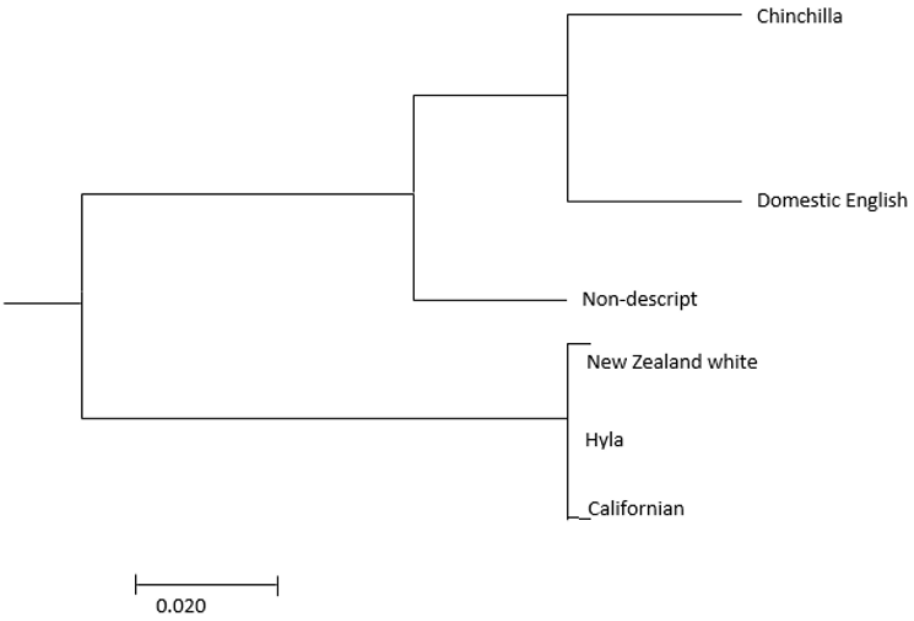

Fig 2: Evolutionary relationships of the population inferred by MEGA 5 program: neighbor-joining phylogenetic tree, derived from mtDNA sequence multiple alignment

CONCLUSION AND RECOMMENDATION

In conclusion, the findings of the current underscores the significance of preserving and harnessing the genetic diversity of locally adapted rabbit genotypes, particularly the non-descript rabbits. Non-descript rabbits displayed higher genetic diversity, reflected in a greater number of haplotypes and higher nucleotide diversity. These genetic traits position them as valuable resources for future breeding programs and sustainable management of genetic variants. Also, the genetic distinctiveness of non-descript rabbits highlights their potential role in maintaining indigenous genetic resources. It is

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recommended that further studies should incorporate nuclear DNA markers and quantitative traits to provide a more comprehensive genetic characterization of rabbit populations in Nsukka. This would facilitate the development of effective breeding strategies and conservation policies.

Acknowledgment

The authors are highly grateful to the Nsukka local farmers who keep and preserve these animal genetic resources and the financial assistance of Nonso Uche (a.k.a Sir DJ Oreo). The lab staff's in African Bioscience with the lab work is also acknowledged by the authors.

Declarations

Ethical approval

A butterfly syringe designed for capillary blood collection was used to draw blood from the lateral marginal ear vein. When handling the rabbit to take blood samples, no physical harm was done to it. The University of Nigeria, Nsukka's institutional ethics directorate examined and authorized our research, finding no evidence of unethical behavior.

Statements of consent

All authors approved of the manuscript's publication.

Competing interests

The authors have no competing interests to disclose.

Authors' contribution

Ikeh, N. E: conception of the work, analysis of data, and drafting of the manuscript

Anizoba, N.W: acquisition of data and drafting of the manuscript

Ugwu, O.G: interpretation of data

Fekurumoh, S.O: acquisition of data

Amaefule, B.C: acquisition of data

Machebe, N.S: supervision and design of the work

Foleng, H.M.: supervision, design of the work and reviewed the manuscript

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