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Research Article

Evaluation of Genetic Structure and Connectivity among Populations of Macrozoobenthic Snail (*Mutela rubens*) from Freshwater Ecosystems in Kano State, Nigeria

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ABSTRACT
Macrozoobenthic snails are important bioindicators and fish food organisms in freshwater ecosystems, yet population genetic data for many Nigerian freshwater molluscs remain scarce. This study evaluated the genetic structure and connectivity among populations of the macrozoobenthic freshwater snail *Mutela rubens* from River Kano, River Bagwai and River Karaye in Kano State, Nigeria, using Random Amplified Polymorphic DNA (RAPD) markers. A total of 96 specimens were analyzed and genetic variation was assessed using five RAPD primers, which generated 82 reproducible loci. Genetic polymorphism ranged from 56.10% to 63.41%, with the highest variation recorded in River Kano (63.41%), followed by River Bagwai (59.76%) and River Karaye (56.10%). Genetic diversity indices revealed higher heterozygosity in River Kano (0.205), while River Karaye recorded the lowest value (0.182), indicating differences in genetic variability and population connectivity. Nei's genetic distance analysis showed moderate genetic differentiation among populations, with values ranging from 0.082 to 0.136, while genetic identity ranged from 0.873 to 0.921. Molecular analysis of variance revealed that 72% of the total genetic variation occurred within populations, whereas 28% was attributed to differences among populations. UPGMA dendrogram grouped the three populations into closely related clusters without genetic outliers. The findings demonstrate that *M. rubens* populations in Kano State freshwater ecosystems possess considerable genetic diversity and connectivity, although localized environmental factors may influence their genetic structuring.

Keywords: Freshwater; Genetic diversity; Mollusc; Population connectivity; RAPD markers

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INTRODUCTION

Freshwater macrozoobenthic molluscs are among the most ecologically important organisms inhabiting inland aquatic ecosystems. They contribute significantly to ecosystem functioning through sediment bioturbation, nutrient cycling, organic matter decomposition, water filtration and energy transfer within benthic food webs. Their intimate association with bottom sediments and sensitivity to changes in water quality also make them valuable bioindicators of environmental health (Chakraborty *et al.*, 2022). Consequently, the persistence and ecological performance of freshwater mollusc populations are essential for maintaining ecosystem stability and the sustainable functioning of freshwater environments.

Among these organisms, *Mutela rubens* is an important freshwater macrozoobenthic mollusc widely distributed in African inland waters. The species performs vital ecological functions through its filtration activities, nutrient recycling and interactions with other benthic organisms, thereby contributing to water quality improvement and ecosystem productivity. In addition to its ecological importance,

M. rubens represents a valuable component of freshwater biodiversity. However, populations of freshwater molluscs are increasingly threatened by anthropogenic disturbances, including agricultural runoff, industrial pollution, habitat degradation, overexploitation and alteration of natural hydrological regimes. These stressors can reduce population sizes, limit dispersal, fragment habitats and ultimately compromise the long-term persistence of natural populations (Onyango, 2024; Liu *et al.*, 2025).

The ability of populations to persist under changing environmental conditions depends not only on their abundance but also on their genetic composition. Genetic structure refers to the distribution of genetic variation within and among populations, while population connectivity, inferred from patterns of gene flow, reflects the extent to which populations exchange individuals and alleles. Together, these parameters determine evolutionary potential, adaptive capacity and resilience to environmental disturbances. Populations that maintain high genetic diversity and effective connectivity are generally better able to adapt to environmental change, whereas genetically isolated populations are more susceptible to inbreeding, loss of genetic variation and local extinction (Moruf and Adekoya, 2020). Consequently, understanding genetic structure and connectivity is fundamental to fisheries management, conservation of genetic resources and predicting population responses to environmental change.

Population genetic analyses provide insights into levels of genetic variation, gene flow, population differentiation and historical demographic processes that cannot be adequately resolved using morphological characteristics alone (Suleiman *et al*., 2023). In freshwater molluscs, genetic structure is often shaped by habitat fragmentation, watershed configuration, dispersal limitations and species-specific life-history characteristics. River connectivity, geographical distance and physical barriers can influence the movement of individuals among populations, thereby affecting patterns of genetic differentiation. Studies on freshwater mussels have demonstrated that populations frequently exhibit distinct genetic structures associated with hydrological connectivity and landscape features, emphasizing the importance of molecular approaches in aquatic biodiversity conservation and management (Garrison *et al*., 2021; Faust *et al*., 2025).

Advances in molecular genetics have greatly improved the assessment of population diversity and connectivity in freshwater organisms. Molecular markers, including mitochondrial DNA sequences, microsatellites and genome-wide approaches, have become indispensable tools for identifying genetically distinct populations, estimating levels of gene flow and defining appropriate conservation and management units (Suleiman *et al*., 2023). These techniques provide reliable information on evolutionary relationships among populations and help determine whether observed population differences result from natural dispersal or ecological isolation. Recent genomic studies have further demonstrated that understanding genetic diversity and population structure is essential for developing effective conservation strategies, restoration programmes and sustainable management plans for vulnerable freshwater mollusc populations (Garrison *et al*., 2021; Gladstone *et al.*, 2022; Faust *et al.*, 2025).

Nigeria possesses extensive freshwater ecosystems comprising rivers, reservoirs, dams and wetlands that support diverse macrozoobenthic communities with considerable ecological and socioeconomic importance (Adamu *et al.*, 2025; Akindele *et al*., 2026). Kano State, located within the Sudan Savannah ecological zone, contains several freshwater ecosystems that sustain populations of *M. rubens* and other benthic organisms despite increasing anthropogenic pressures associated with urbanization, agriculture, water abstraction and environmental pollution. These ecosystems provide an appropriate natural setting for investigating patterns of genetic diversity and connectivity among freshwater mollusc populations inhabiting different but potentially interconnected aquatic habitats.

Although studies on freshwater molluscs have increasingly employed molecular tools to investigate population genetic diversity and connectivity in different parts of the world, similar information remains scarce for Nigerian freshwater species. Existing studies in Kano State have demonstrated the usefulness of molecular techniques for revealing genetic variation and population relationships among freshwater snail populations, suggesting that substantial microgeographic genetic structuring may occur even among populations inhabiting relatively connected freshwater systems (Moruf and Muhammad, 2023; Onyango, 2024). Nevertheless, there remains little information regarding the genetic structure, population differentiation and connectivity of *M. rubens* across freshwater ecosystems in Kano State. This knowledge gap limits the development of evidence-based conservation strategies and sustainable management programmes for the species and the freshwater ecosystems it inhabits.

Therefore, this study was undertaken to evaluate the genetic structure and connectivity among populations of *Mutela rubens* inhabiting selected freshwater ecosystems in Kano State, Nigeria. Specifically, the study aims to assess the genetic diversity within and among populations, determine the extent of population differentiation and gene flow, and evaluate patterns of connectivity among freshwater populations. The findings will provide baseline genetic information for the conservation of *M. rubens*, support sustainable management of freshwater biodiversity and contribute to understanding how freshwater mollusc populations may respond to ongoing environmental changes in Nigerian inland waters.

MATERIALS AND METHODS

Study Area

The study was conducted in selected freshwater ecosystems of Kano State, Nigeria, namely River Kano, River Karaye and River Bagwai (Figure 1). The approximate geographical coordinates of the sampling sites were 11°50′45″ N, 8°30′21″ E for River Kano, 11°45′45″ N, 8°00′35″ E for River Karaye, and 12°06′00″ N, 8°08′37″ E for River Bagwai. Kano State is located within the Sudan savannah ecological zone of northern Nigeria and is characterized by seasonal freshwater systems that support fisheries, agricultural activities and diverse aquatic organisms (Aliyu *et al.*, 2021). The selected rivers represent important freshwater habitats influenced by anthropogenic activities including irrigation, domestic use, fishing activities and agricultural runoff. River Karaye is associated with the Challawa–Kano River system, which contributes significantly to water supply, irrigation and fisheries development in the region. River Bagwai is located within the northern agricultural landscape of Kano State where farming and aquatic resource utilization are common livelihood activities.

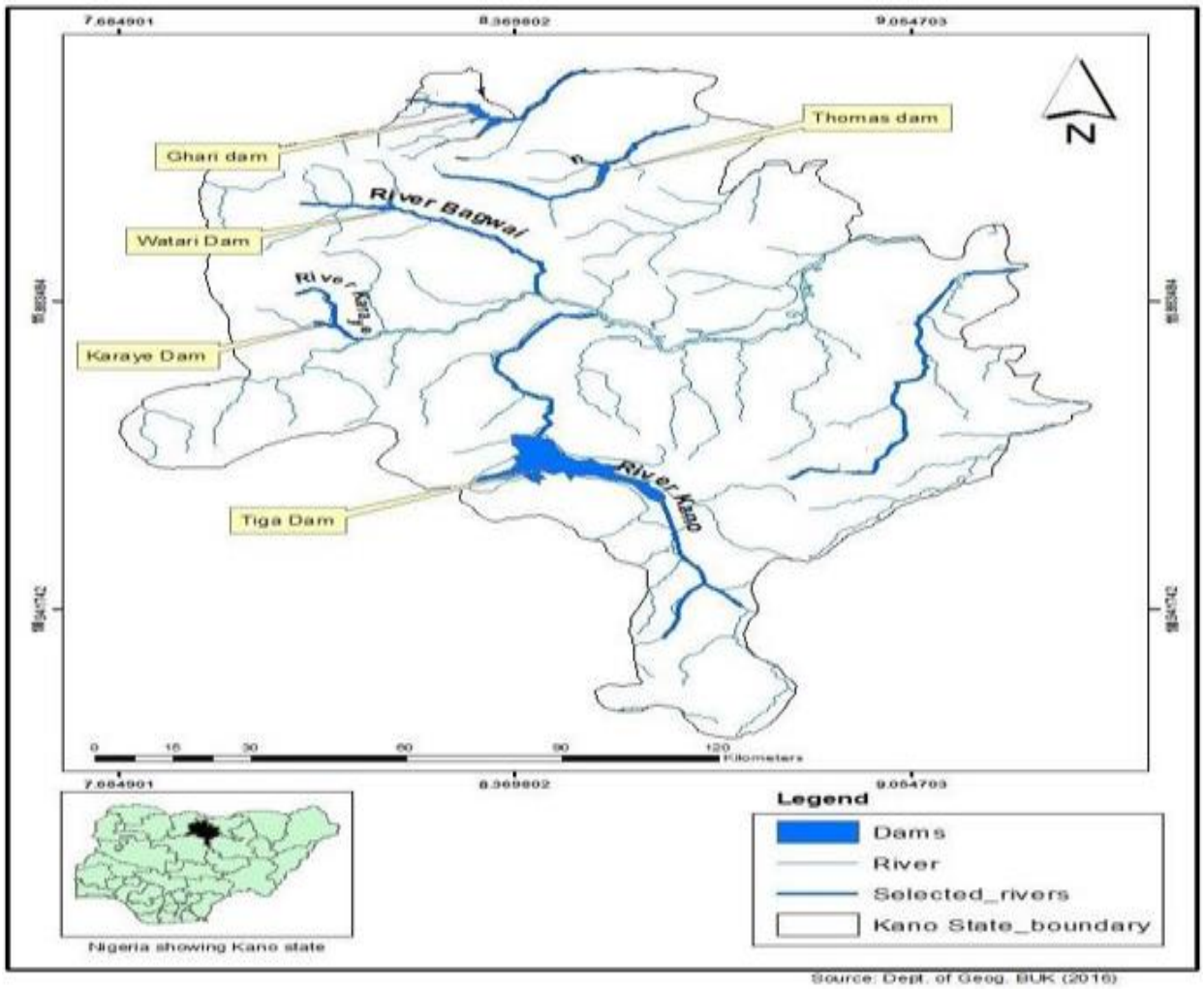

Figure 1: The map of Kano state with the sampling locations (modified from Suleiman, 2017)

Sample Collection and Identification

A total of 96 live specimens of the freshwater macrozoobenthic snail, *M. rubens*, were collected from the spatially distributed freshwater ecosystems using a standard aquatic snail scoop, after which the collected materials were washed and individual specimens were manually sorted. The recovered specimens were carefully transferred into pre-labelled plastic containers and transported to the Laboratory of the Department of Fisheries and Aquaculture, Bayero University, Kano, for further processing.

Species identification was carried out based on external morphological characteristics including shell shape, shell size, colouration and other diagnostic features following the identification guide for African freshwater molluscs by Kristensen (1987). Specimens intended for molecular analysis were preserved in 70% ethanol and subsequently subjected to molecular characterization in collaboration with Precision Laboratory Ltd., Ibadan, Oyo State, Nigeria.

Laboratory Molecular Analysis

Genomic DNA Extraction

Genomic DNA was extracted from the soft tissue of *M. rubens* using the Genomic DNA Tissue MiniPrep Kit following the procedure described by Winnepenninckx *et al.* (1993). Tissue samples were carefully dissected using sterilized instruments to avoid contamination before DNA extraction. The extracted DNA was preserved at 4°C until further molecular analysis.

The quantity and quality of extracted DNA were determined using a Nanodrop spectrophotometer (NANO 1000, China). Approximately 1 µL of each DNA extract was placed on the spectrophotometer platform, with distilled water used as the blank for calibration. DNA concentration was estimated based on absorbance at 260 nm and expressed as ng/µL. The purity of the extracted DNA was evaluated using the absorbance ratio at 260 nm/280 nm, which indicates DNA quality and suitability for downstream molecular analysis.

PCR Amplification and RAPD Analysis

Polymerase Chain Reaction (PCR) amplification was performed in a 50 µL reaction mixture containing PCR buffer (50 mM KCl, 0.1% Triton X-100, 10 mM Tris-HCl pH 8.3, and 1.5 mM $MgCl_2$), 2.5 mM dNTPs (BioBasic, Canada), 5.0 µM of RAPD primer, 50 ng template DNA, and 3 U Taq DNA polymerase following the protocol described by Simpson *et al.* (1993). Five randomly selected RAPD primers obtained from laboratory stocks were used for amplification of genomic DNA fragments. The primers included CTGCTGGGAC, AGGGAACGAG, GTGAGGCGTC, GTTGCCAGCC and TGCCGAGCTG.

Amplification was carried out using a thermal cycler (Hamburg, Germany) under the following cycling conditions: initial denaturation at 94°C for 4 minutes, followed by 35 amplification cycles consisting of denaturation at 94°C for 1 minute, annealing at 36°C for 1 minute, and extension at 72°C for 1 minute. A final extension step was conducted at 72°C for 10 minutes.

Agarose Gel Electrophoresis and Visualization

The amplified PCR products were separated using agarose gel electrophoresis. Agarose gel was prepared at 1.8% concentration in 1× TBE buffer containing 0.9 M Tris, 0.9 M boric acid, and 20 mM EDTA at pH 8.3. DNA samples were mixed with loading buffer and electrophoresed at 100 V for four hours. A 100 bp molecular weight marker was used as a reference for estimating fragment sizes.

Following electrophoresis, the agarose gels were stained with ethidium bromide (0.5 µg/mL) for visualization of amplified DNA fragments. The resulting RAPD profiles were photographed under ultraviolet illumination using a digital imaging system.

Data Analysis

The RAPD-PCR banding profiles generated from *M. rubens* specimens were analyzed using Phylip software (version 2.1, USA). Amplified fragments were scored based on the presence (1) or absence (0) of reproducible DNA bands to generate a binary data matrix. Electrophoretic profiles were converted into binary matrices using PyElph version 1.4. Population genetic analyses were performed using GenAlEx version 6.5 to estimate genetic diversity and differentiation. Genetic relationships among populations were determined using clustering analysis, and a phylogenetic tree was constructed using the Unweighted Pair Group Method with Arithmetic Mean (UPGMA) approach in MEGA version 11.

RESULT

RAPD Polymorphism Profile

The five RAPD primers successfully amplified genomic DNA from 96 individuals of *M. rubens* (Table 1). A total of 82 reproducible loci were generated from the RAPD profiles, with polymorphic loci ranging from 56.10% to 63.41% across populations. The relatively high percentage of polymorphic bands observed among the populations indicates considerable genetic variation within the studied *M. rubens* populations. The highest level of genetic polymorphism was recorded in the River Kano population (63.41%), followed by River Bagwai

(59.76%), while the lowest polymorphism was observed in River Karaye (56.10%).

RAPD Band Frequency and Mean Heterozygosity

The total RAPD banding patterns generated from binary (diploid) data among *M. rubens* populations are presented in Table 2 and Figure 2. The number of amplified bands with allele frequency ≥5% varied among the sampled populations, ranging from 47 bands in River Karaye, 50 bands in River Bagwai and 55 bands in River Kano. The observed variation in band frequencies indicates differences in genetic composition among the studied populations. Mean heterozygosity values also followed a similar pattern, with River Karaye recording the lowest genetic diversity (0.182), followed by River Bagwai (0.196), while River Kano exhibited the highest heterozygosity value (0.205).

Table 1: RAPD Polymorphism Profile among *Mutela rubens* Populations from Freshwater Ecosystems in Kano State, Nigeria

Population	No. of Individuals Analysed	Total Loci Scored	No. of Polymorphic Loci	Polymorphic Bands (%)
River Kano	32	82	52	63.41
River Bagwai	32	82	49	59.76
River Karaye	32	82	46	56.1
Overall	96	82	49	59.76

Table 2: RAPD band frequency and mean heterozygosity among *Mutela rubens* populations from freshwater ecosystems in Kano State, Nigeria

Population	Number of Bands (Frequency ≥ 5%)	Mean Heterozygosity
River Karaye	47	0.182
River Bagwai	50	0.196
River Kano	55	0.205

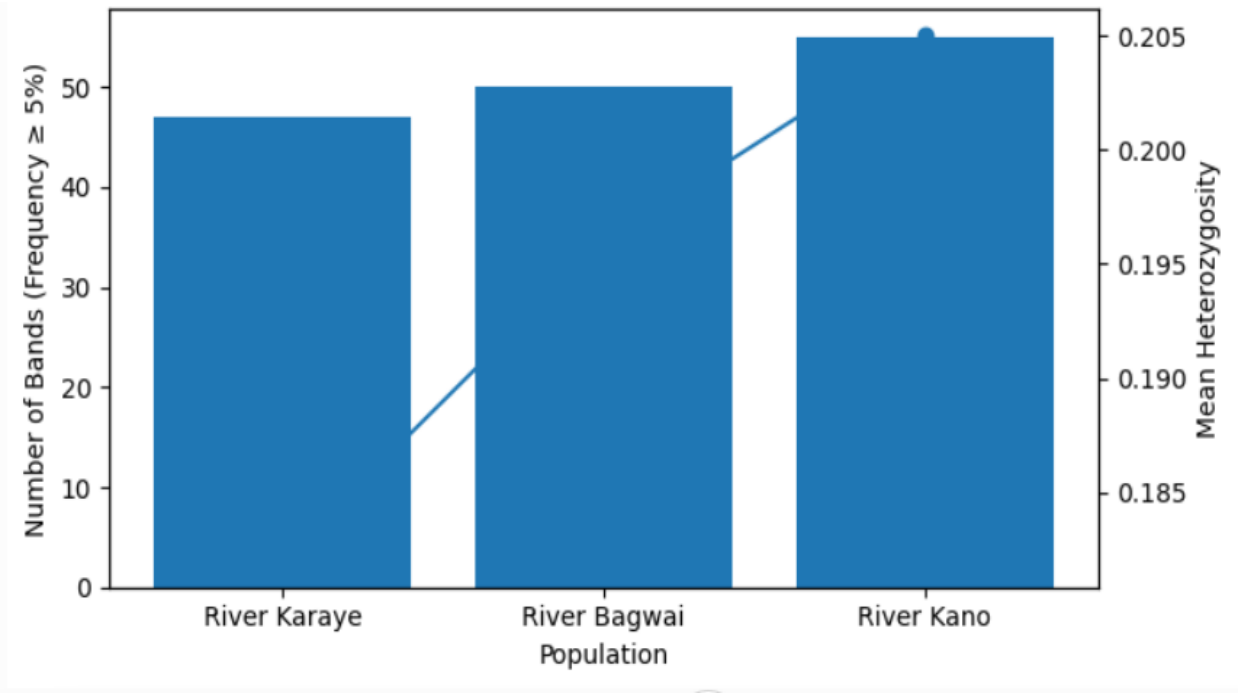

Figure 2: Total band patterns for binary (diploid) data by populations

Genetic Differentiation and Connectivity

Estimates of Nei's genetic distance revealed moderate genetic differentiation among the three *M. rubens* populations, indicating detectable genetic structuring across the freshwater ecosystems studied (Table 3). The highest genetic distance was recorded between the River Kano and River Bagwai populations (0.136), suggesting greater genetic separation and possible reduced gene exchange between these populations. Conversely, the lowest genetic distance

was observed between River Kano and River Karaye populations (0.082), indicating closer genetic similarity and stronger connectivity between these two populations. Genetic identity values ranged from 0.873 to 0.921, with the highest genetic identity observed between River Kano and River Karaye (0.921), while the lowest identity occurred between River Kano and River Bagwai (0.873).

The analysis of molecular variation revealed that genetic diversity within *M. rubens* populations accounted for 72% of the total genetic variation, while variation among populations contributed 28% (Figure 3). The high proportion of within-population variation indicates substantial genetic diversity among individuals and suggests active gene exchange and connectivity within the freshwater ecosystems studied. The moderate genetic differentiation among River Kano, River Bagwai and River Karaye populations implies that geographical separation and localized environmental conditions may contribute to population structuring, although genetic isolation is not pronounced.

The UPGMA dendrogram constructed based on Nei's genetic distance revealed the formation of three closely related clusters corresponding to *M. rubens* populations from River Kano, River Bagwai, and River Karaye (Figure 4). The clustering pattern showed no distinct genetic outlier, indicating that all populations share considerable genetic similarity. River Kano and River Bagwai populations formed a closer genetic association, suggesting higher connectivity and possible gene flow between these freshwater habitats. The River Karaye population, although forming a separate branch, remained genetically related to the other populations, reflecting shared ancestry and historical connectivity among the sampled ecosystems.

Table 3: Pairwise Population Nei Genetic Values of *M. rubens* from three water bodies in Kano

Population Comparison	Nei's Genetic Distance	Genetic Identity
River Kano vs River Karaye	0.082	0.921
River Kano vs River Bagwai	0.136	0.873
River Karaye vs River Bagwai	0.094	0.910

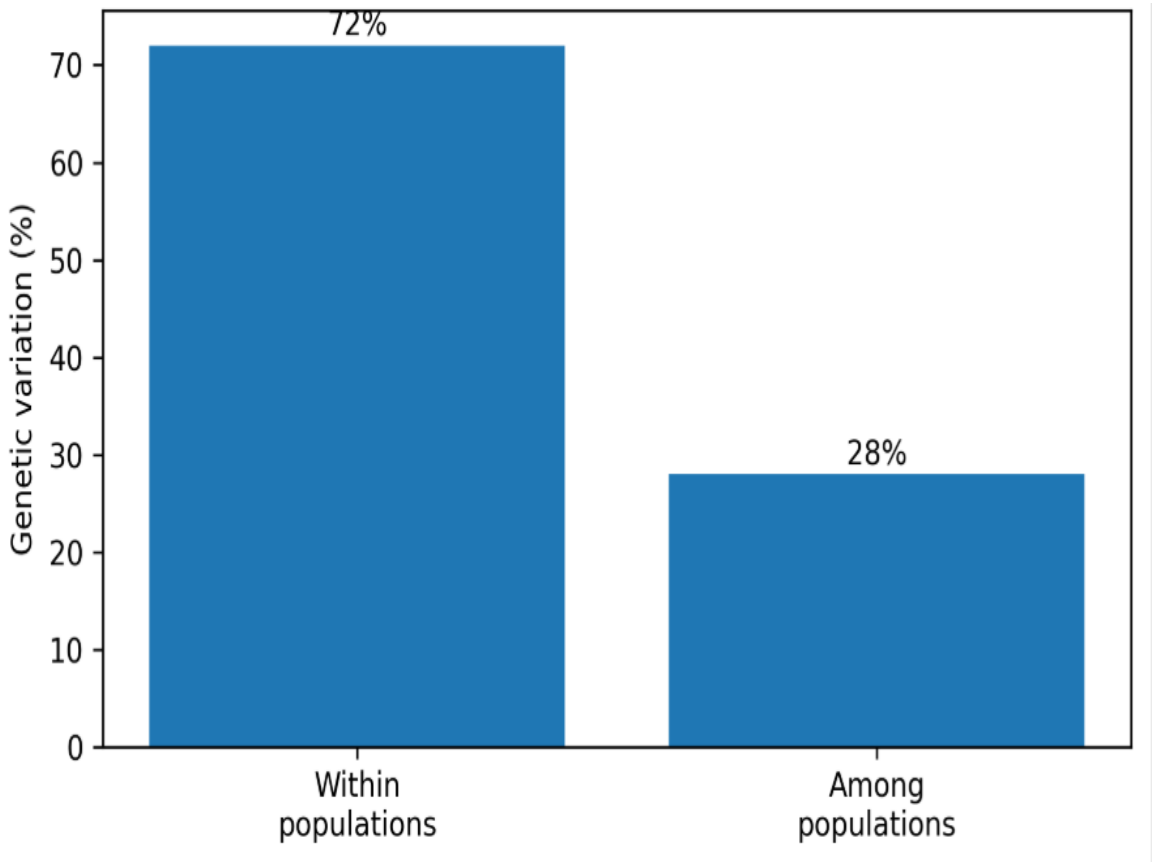

Figure 3: Genetic variation partitioning in *M. rubens* populations from three water bodies in Kano, Nigeria

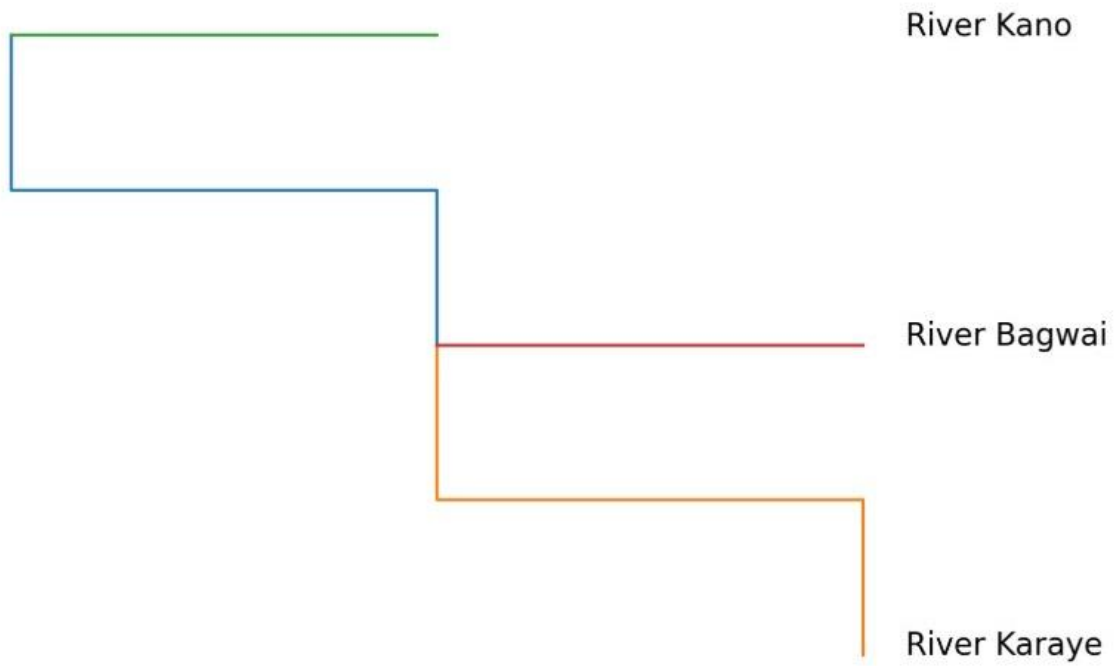

Figure 4: UPGMA dendrogram showing population relationship (Nei's genetic distance)

DISCUSSION

The high RAPD polymorphism observed among *M. rubens* populations indicates substantial genetic variability, suggesting that the species possesses a broad genetic base capable of supporting adaptation to varying environmental conditions. High levels of polymorphism are generally regarded as evidence of extensive allelic diversity and have frequently been reported in freshwater molluscs inhabiting interconnected aquatic systems where dispersal facilitates gene exchange among populations (Martin *et al.*, 2020). Such genetic diversity is essential for maintaining evolutionary potential and increasing resilience to environmental perturbations, habitat modification and climate change (Schierenbeck 2017; Gaitán-Espitia and Hobday, 2021). The higher polymorphism recorded in River Kano suggests enhanced population connectivity, larger effective population size and greater habitat heterogeneity, supporting reports by Moruf and Adekoya (2018) that environmental complexity promotes genetic variation in aquatic invertebrates. Similar observations have been reported for freshwater mussels, where habitat connectivity and stable hydrological conditions facilitate the maintenance of high genetic diversity through continuous gene flow (Shahnaseri *et al.*, 2026). Conversely, the relatively lower diversity observed in River Karaye may reflect restricted gene exchange, reduced effective population size or localized selective pressures, as noted by Alther *et al.* (2021), where reduced connectivity contributes to population differentiation in freshwater organisms. Habitat fragmentation has been widely recognized as a major driver of declining genetic diversity in freshwater fauna because it limits dispersal and increases the effects of genetic drift (Shahnaseri *et al.*, 2026).

The variation in RAPD band frequencies and heterozygosity among *M. rubens* populations further indicates differences in genetic diversity and population connectivity across the studied freshwater ecosystems. Heterozygosity is widely regarded as an important indicator of adaptive potential and long-term population viability because genetically diverse populations are generally more capable of responding to environmental change (Schierenbeck 2017; Gaitán-Espitia and Hobday, 2021). The higher heterozygosity observed in River Kano suggests greater genetic exchange among individuals, which agrees with the findings of Schierenbeck (2017) that populations with higher genetic variability often experience increased gene flow and connectivity. Similarly, Shila *et al.* (2026) reported that RAPD-based polymorphism effectively reveals population-level genetic differentiation. Comparable findings have been reported in freshwater bivalves and gastropods where molecular markers demonstrated that populations occupying

hydrologically connected habitats exhibit higher heterozygosity than isolated populations (Moruf and Muhammad, 2023; Onyango, 2024). The lower diversity in River Karaye may reflect habitat isolation, reduced effective population size or restricted dispersal, consistent with Pironon *et al.* (2017), who emphasized the role of geographical separation in shaping population genetic structure. Reduced connectivity among freshwater habitats has been shown to decrease allelic richness and increase genetic differentiation through restricted migration and localized genetic drift (Machado *et al.*, 2022).

Nei's genetic distance values observed among *M. rubens* populations indicate moderate genetic differentiation, suggesting that both geographic separation and habitat connectivity influence population structure. Moderate genetic distances generally indicate that populations remain evolutionarily related despite experiencing some degree of restricted gene flow (Combs *et al.*, 2019). Similar patterns were reported by Schierenbeck (2017), who noted that genetic distance reflects the extent of evolutionary divergence among populations. The closer genetic relationship between River Kano and River Karaye populations agrees with findings of Martin *et al.* (2020), where aquatic organisms occupying more connected habitats often exhibit higher genetic similarity due to continued gene flow. Similar observations have been reported in freshwater mussels where river connectivity plays a fundamental role in maintaining genetic homogeneity among neighbouring populations (Moruf and Muhammad, 2023). Conversely, the greater differentiation between River Kano and River Bagwai may indicate reduced dispersal opportunities, hydrological barriers or localized environmental conditions that limit gene exchange, as reported for freshwater molluscs by Benhamdoun *et al.* (2024). Such differentiation may also reflect the combined influence of genetic drift, habitat fragmentation and environmental selection acting on geographically separated populations (Gaitán-Espitia and Hobday, 2021).

The high within-population genetic variation and comparatively moderate among-population variation observed in the present study indicate that *M. rubens* populations retain considerable genetic diversity while maintaining appreciable connectivity across the freshwater ecosystems investigated. High within-population variation is a characteristic feature of many freshwater mollusc populations and is generally associated with large effective population sizes, historical connectivity and sufficient gene flow to counteract the effects of genetic drift (Martin *et al.,* 2020). Similar patterns have been reported in several freshwater mussel species, where most genetic variation occurs within rather than among populations despite moderate geographic separation (Onyango, 2024). The clustering of populations without pronounced genetic separation agrees with findings of Schröder *et al.* (2022), who reported close genetic relationships among aquatic invertebrate populations inhabiting connected freshwater systems. Although some differentiation was detected, it likely reflects localized environmental influences and varying degrees of habitat isolation rather than complete reproductive isolation. The observed genetic similarity suggests historical and contemporary connectivity among freshwater habitats in Kano State, allowing continued exchange of genetic material among populations. Such connectivity is essential for maintaining adaptive capacity and should be considered in future conservation and management strategies aimed at preserving the genetic integrity and long-term sustainability of *M. rubens* populations (Schierenbeck 2017; Gaitán-Espitia and Hobday, 2021).

CONCLUSION

The study confirms substantial genetic variation and connectivity among *Mutela rubens* populations from freshwater ecosystems in Kano State, Nigeria. High within-population variation and moderate differentiation among populations indicate active gene flow and limited genetic isolation. The observed genetic structure suggests that habitat connectivity and environmental conditions influence population dynamics, providing important baseline information for conservation and sustainable management of freshwater macrozoobenthic resources.

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