GENETIC SIMILARITY AND GENE FLOW IN FRESHWATER SNAIL, *BULINUS GLOBOSUS* POPULATIONS FROM SELECTED NATURAL HABITATS IN KANO STATE (NIGERIA)

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ABSTRACT

The population genetic structure of *Bulinus globosus*, an important intermediate host snail for *Schistosma haematobium*, in Nigeria was studied using Random Amplified Polymorphic DNA Technique. The five primers amplified genomic DNA of *B. globosus* from three populations with one region and 999 permutations. With 79 loci, the % polymorphic bands for each primer across all population samples were 55.70% (River Karaye), 58.23% (River Bagwai) and 60.76% (River Kano). The mean heterozygosity was 0.175, 0.190 and 0.197 for snails caught in Karaye, Bagwai, and Kano respectively. The highest genetic distance (0.141) and identity (0.919) were observed between the populations of Bagwai, Kano, and Karaye and Bagwai. The variations within and among the snail populations were 76% and 4% respectively, while the UPGMA dendogram revealed no outliers. The gene pool of *B. globosus* was microgeographically fragmented.

RÉSUMÉ: Similarité génétique et flux de gènes chez des populations d'escargot d'eau douce, *Bulinos globosus*, issues d'habitats sélectionnés dans l'État de Kano, Nigéria.

La structure génétique de *Bulinus globosus*, un escargot hôte intermédiaire important pour *Schistosma haematobium*, a été étudiée au Nigeria à l'aide de la technique d'amplification aléatoire d'ADN polymorphe. Les cinq amorces amplifiées d'ADN génomique de *B. globosus* à partir de trois populations avec une région et 999 permutations. Avec 79 loci, le pourcentage de bandes polymorphes pour chaque amorce dans l'ensemble des échantillons de population était de 55,70% (Karaye), 58,23% (Bagwai) et 60,76% (Kano). L'hétérozygotie moyenne était de 0,175, 0,190 et 0,197 pour les escargots capturés dans la rivière Karaye, la rivière Bagwai et la rivière Kano. La plus grande distance génétique (0,141) et d'identité (0,919) ont été observées entre les populations de la Bagwai et Kano, et entre la Karaye et la Bagwai. Les variations au sein des populations d'escargots étaient de 76% et 24% respectivement, tandis que le dendrogramme UPGMA ne révélait pas de valeurs aberrantes. Le pool génique de *B. globosus* était fragmenté à l'échelle micro-géographique.

REZUMAT: Asemănarea genetică și fluxul de gene la melcul de apă dulce, populația *Bulinus globosus* din habitate naturale selectate din statul Kano, Nigeria.

Structura genetică a populației de *Bulinus globosus*, un melc gazdă intermediar important pentru *Schistosma haematobium*, din Nigeria a fost studiată folosind Tehnica de Amplificare Aleatorie a ADN-ului polimorf. Cei cinci primeri au amplificat ADN-ul genomic al *B. globosus* de la trei populații cu o regiune și 999 permutări. Cu 79 de loci, procentajul benzilor polimorfe pentru fiecare primer din toate probele populației au fost de 55,70% (râul Karaye), 58,23% (râul Bagwai) și 60,76% (râul Kano). Heterozgozitatea medie a fost de 0,175, 0,190 și 0,197 pentru melcii din râul Karaye, râul Bagwai și, râul Kano. Cea mai mare distanță genetică (0,141) și identitate (0,919) au fost observate între populațiile Bagwai și Kano și, Karaye și Bagwai. Variațiile în interiorul și între populațiile de melci au fost de 76% și, respectiv, 24%, în timp ce Dendograma UPGMA a arătat fără valori aberante. Baza genetică a *B. globosus* a fost fragmentată microgeografic.

INTRODUCTION

Current rates of species loss have triggered numerous attempts to protect and conserve biodiversity (Wäldchen et al., 2018). Species conservation, however, requires species identification tools, a competence obtained through molecular technique. Accurate species identification is the basis for all aspects of taxonomic research and is an essential component of workflows in biological research (Wäldchen and Mäder, 2018). Biologists are asking for more efficient methods to meet the identification demand. Meanwhile, information on the genetic structure of fish is useful for optimizing identification of stocks, stock enhancement, breeding programs, management of sustainable yield and preservation of genetic diversity (Sultana et al., 2018).

DNA polymorphisms have been extensively employed to assess genetic diversity in aquatic organisms (Phillips et al., 2019). Randomly amplified polymorphic DNA (RAPD) fingerprinting offers a rapid and efficient method for generating a new series of DNA markers in fish (SriHari et al., 2022). RAPD analysis is a technique based on the polymerase chain reaction (PCR) amplification of discrete regions of genome with short oligonucleotide primers of an arbitrary sequence (SriHari et al., 2022). This method is simple and quick to perform; most importantly, no prior knowledge of the organism's genetic make-up is required. This technique has been used extensively to detect genetic diversity in crabs (Moruf and Adekoya, 2020), lobsters (Jeena et al., 2016) and fish (Suleiman et al., 2023). It has also been used to evaluate genetic diversity in various snail species such as *Galba schirazensis* (Lounnas et al., 2018), *Biomphalaria pfeifferi* (Tian-Bi et al., 2019; Manyangadze et al., 2021), *Oncomelania hupensi*s (Qiu et al., 2019) and *Hydrobioides nassa* (Bunchom et al., 2021).

The determination of taxa is particularly important in the case of organisms involved in spreading diseases. Indeed, prophylactic strategies require a thorough knowledge of the biology and ecology of parasites and their vectors. Schistosomiasis caused by *Schistosma haematobium* and *S. mansoni* is a parasitic disease commonly found in tropical and subtropical regions and is considered as the third most important tropical disease after malaria and intestinal helminthiasis (WHO, 2014).

The freshwater snail *B. globosus* is an important intermediate host for *S. haematobium*, the causative agent of urinary schistosomiasis in tropical and sub-tropical countries (Bórquez et al., 2020). This disease is of major health concern, especially in Africa where most cases have been reported. In 2012 alone it was reported that an estimated 42.1 million people were treated for this disease, and 249 million required preventative treatment, of which 90% lived in Africa (WHO, 2014). *Bulinus* spp. are hermaphrodites that are capable of selfing or outcrossing, with different species adopting either selfing or outcrossing as the preferential reproductive mode (Keeney and Yurco, 2021). Limited gene flow encourages inbreeding and this is probably the most significant factor shaping the genetic structure of *B. globosus*, which normally has a mixed reproductive strategy. However, it adopts only one reproductive mode at any particular time, with evidence proposing outcrossing as a way to avoid inbreeding depression (Qiu et al., 2019; Koene, 2021).

The common habitats of *B. globosus* are shallow waters near shores of lakes, ponds, streams and irrigation channels (Manyangadze et al., 2021). The important factors that shape the genetic structure and spatial distribution of snails include the distribution of habitats, which is influenced by the spatial and temporal fluctuations in water availability (Bórquez et al., 2020). Habitats also vary in space with regards to environmental factors such as predation, parasitism, and competition (Pyron and Brown, 2015). Parasites have also been found to have an effect on the genetic diversity, reproduction mode and overall structure of freshwater snail populations (Tian-Bi et al., 2019). In Nigeria, a limited number of molecular techniques have

been utilized to study the genetic structure of African freshwater snail populations. Therefore, a deeper understanding of the geographical distribution and population structure of *B. globosus* is crucial in understanding its population genetic diversity and fitness because the species plays a major role as a vector of schistosomiasis. Hence, the present study was carried out to ascertain the genetic similarity and gene flow in the freshwater snail populations from selected natural habitats in Kano State using versatile RAPD markers.

MATERIAL AND METHODS

Study area

The study was conducted in three selected rivers in Kano State, Nigeria. The river Kano is located in the South while the Rivers Karaye and Bagwai are in the western part of the state (Fig. 1). The locations were selected due to their same origin. Kano State is located in the semiarid area of Northwestern Nigeria. It has a population of 9,383,682 comprising of 4,844,128 males and 4,539,534 females (NPC, 2006). Kano State is the commercial nerve centre of Northern Nigeria. It is located between latitude 10°33' and 12°27'North of the equator and longitude 7°34' and 9°29' East of the Greenwich meridian and as such it is part of Sudano-Sahelian vegetation zone of Nigeria.

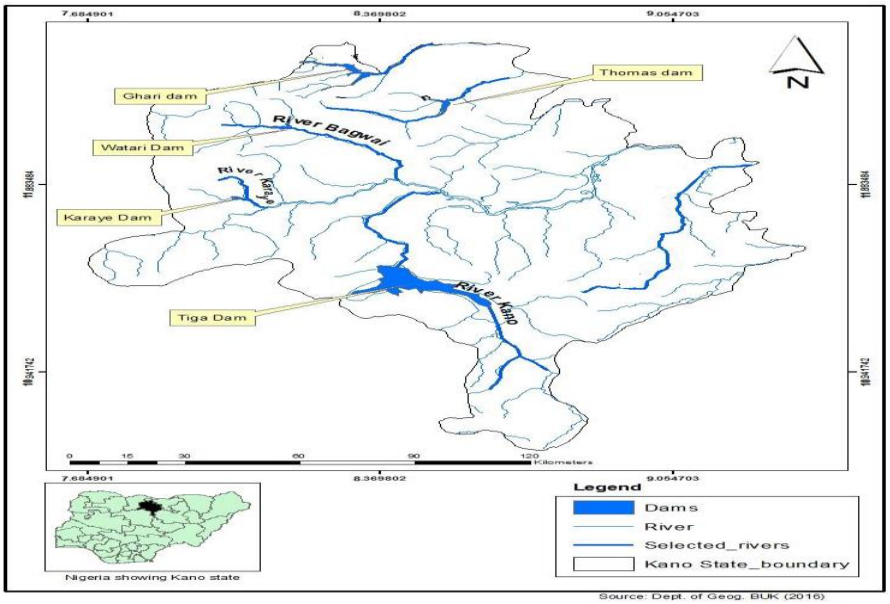

Figure 1: The map of Kano state with the sampling locations; modified from Suleiman (2017).

Sample collection and preparation

A total of 185 specimens of *B. globosus* were collected from spatially distributed water contact sites in the study area in 2023. The specimens were collected alive using a standard snail scoop, the contents washed, and the snails picked manually. The recovered snails were transported in pre-labeled plastic containers to the laboratory of the Department of Fisheries and Aquaculture, Bayero University, Kano. Each snail was identified based on its morphological characteristics using the field guide to African freshwater snails (Kristensen, 1987). The samples for molecular studies were preserved in 70% ethanol and further analyzed in conjunction with the African Bioscience Ltd in Ibadan, Oyo State.

Laboratory analysis

DNA was extracted from the head-foot tissue of each snail using the Genomic DNA Tissue MiniPrep Kit as described by Winnepenninckx et al. (1993). The concentration of the extracted DNA was spectrophotometrically estimated. DNA was stored at 4°C until needed. DNA yield was determined with a nanodrop spectrophotometer (NANO 1000, China) based on the maximum absorbance of DNA at 260 nm. One (1) µL of the DNA sample was applied on the platform of the nanodrop spectrophotometer and a reading was taken after adjustment of absorbance to zero using water as blank. The yield was measured in ng/µL. The 260 nm/280 nm ratio was obtained to give an analysis of the purity of the sample and the concentration of the extracted DNA was also found.

The amplification reaction was performed in 50 ml volume mixtures consisting of Polymerase Chain Reaction buffer (50 mM KCl, 0.1% Triton X-100,10 mm Tris-HCl pH 8.3, 1.5 mM MgCl2), 2.5 mM dNTP (BioBasic, Canada), 5.0 µm of each RAPD primers, 50 ng of template DNA and 3U. Taq DNA polymerase with the protocol described by Simpson et al. The five RAPD primers: 1(CTGCTGGGAC), 2(AGGGAACGAG), 3(GTGAGGCGTC), 4(GTTGCCAGCC), and 5(TGCCGAGCTG), used in the PCR reaction were arbitrarily selected from laboratory stocks. Amplifications of DNA fragments were carried out by using a thermal cycler (Hamburg, Germany) with the following cycling profile: pre-denaturation at 94°C for four minutes, followed by 35 cycles of amplification (one min. denaturation at 94°C, one min. annealing at 36°C and one min. extension at 72°C). The process concluded with an extension at 72°C for 10 min. analysis of the resultant amplification products was done at 100 V for four hours with 1.8% agarose gel electrophoresis (BioRAD, USA) using TBE 1 × buffer (0.9 M Tris, 0.9 M Boric acid and 20 mM EDTA, pH 8.3). Furthermore, a DNA size criterion of 100 bp molecular weight marker was used. In order to visualize the amplified products with a digital camera, ethidium bromide was used to stain them.

Agarose gel (1.5 gm/100 ml) was prepared in pH 8.0 buffer which contained 89 mmol of Tris-borate, 2 mmol of EDTA, and 89 mmol of boric acid. After mixing the DNA samples with the loading buffer, they were electrophoresed at 50 volts for one hour. Afterward, agarose gel was stained with ethidium bromide (0.5 µg/ml) for 30 minutes and then photographed on UV light with a digital camera.

Data analysis

The RAPD Polymerase Chain Reaction (PCR) banding patterns generated with the primer were analyzed using Phyllip software (version 2.1, USA). Existence or non-existence of amplicons in each lane of Agarose Gels was premised on scores recorded in binary format. Scores were exclusively allotted only to the intense and reproducible bands that ranged between 400 and 1200 bp. Electrophoresis gels were scored into binary matrix using PyElph 1.4. Genetic analyses were conducted using GenAlEx 6.5 and UPGMA Phylogeny was constructed using MEGA 11.

RESULTS AND DISCUSSION

The five RAPD primers amplified the genomic DNA of 96 individuals of *B. globosus* from three populations (Fig. 2) with one region and 999 permutations. With 79 loci, the percentages of polymorphic bands for each primer across all population samples were 55.70% (River Karaye), 58.23% (R. Bagwai), and 60.76% (R. Kano) suggesting that inbreeding is not a major concern for this species. The study revealed a wide variation of polymorphic loci among the three populations. The highest level of polymorphism (60.76%) was exhibited by the River Kano population, whereas the lowest level of polymorphism (55.70 %) was exhibited by the River Karaye population. The percentage of polymorphic bands in *B. globosus* was

greater than that of the Indian mangrove crab, where the level of polymorphic bands was 24.60% in *Grapsus albolineatu*s (Suresh and Madhuri et al., 2017). Although the sample size from each geographic site in this study was limited, specimens were collected from different geographic locations in Kano State. This should be sufficient to generate the preliminary data on genetic diversity and population differentiation of *B. globosus* in Kano State, Nigeria.

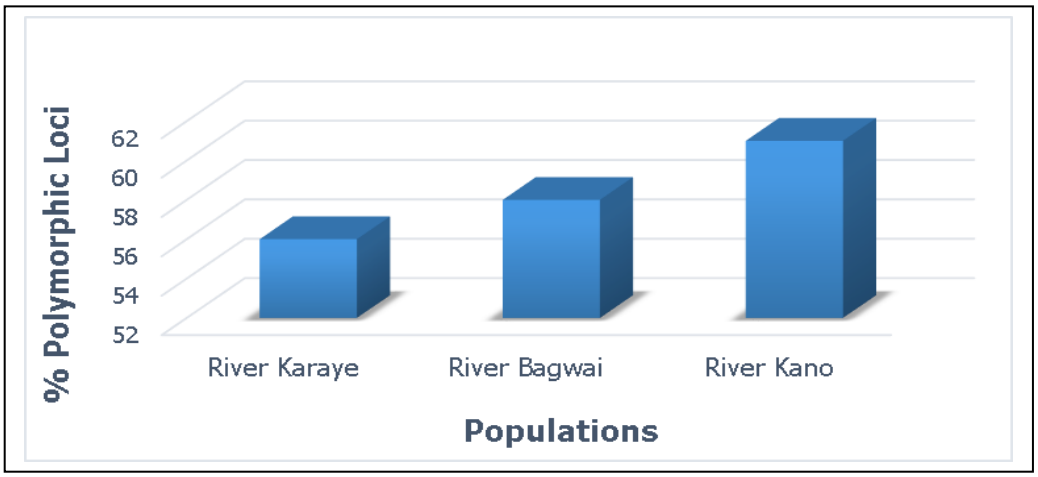

Figure 2: Percentage of Polymorphic Loci.

As indicated by the total band patterns for binary (diploid) data by populations in figure 3, the number of bands (or a number of bands freq. >= 5%) ranges between 45 (River Karaye), 46 (River Bagwai) and 53 (River Kano) while the mean heterozygosity was 0.175, 0.190 and 0.197 for snail caught in River Karaye, River Bagwai and River Kano respectively. The heterozygosity displayed by *B. globosus* is lower than the mean heterozygosity of 0.463 recorded in *Orechromis niloticus* (Mahboob et al., 2019). These population-specific unique bands can be used to detect any possible mixing of these populations, especially during selective breeding programs (Houston et al., 2020). Hassanien and Al-Rashada (2019), Kajungiro et al. (2019), Lounnas et al. (2018), and Hobbs et al. (2021) also observed population-specific bands in *Penaeus semisulcatus*, *Oreochromis niloticus*, *Galba schirazensis*, and *Segmentina nitida* respectively.

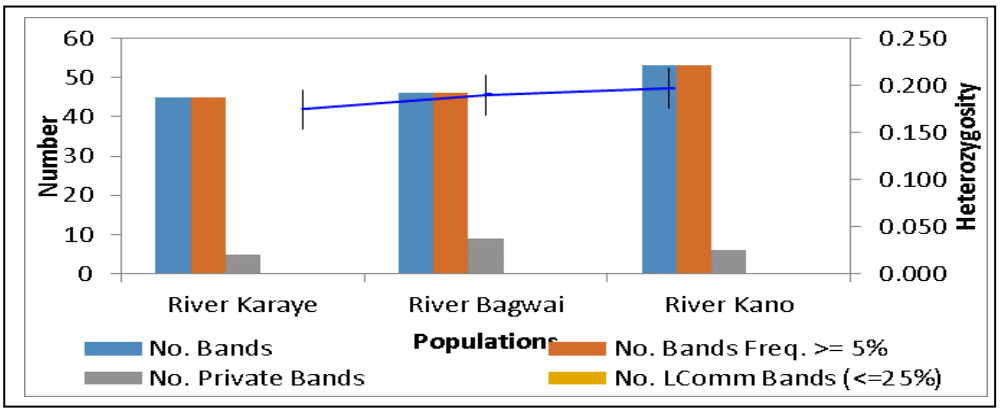

Figure 3: Total band patterns for binary (diploid) data by populations.

Estimates of Nei's genetic distance demonstrated sufficient genetic divergence to discriminate the samples of different populations of *B. globosus* (Tab. 1). The highest genetic distance (0.141) and identity (0.919) was observed between the populations of River Bagwai and River Kano, and River Karaye and River Bagwai, respectively. Generally, the levels of genetic distance between paired geographic samples did not reveal larger genetic distance with greater geographic distance (Moruf and Adekoya, 2020).

Table 1: Pairwise Population Nei Genetic Values of *B. globosus* from three water bodies in Nigeria.

Population 1	Population 2	Nei Distance	Nei Identity
River Karaye	River Bagwai	0.085	0.919
River Karaye	River Kano	0.086	0.918
River Bagwai	River Kano	0.141	0.868

The variations within and among the snail populations are 76% and 24% respectively (Fig. 4) while the UPGMA Dendogram among *B. globosus* populations using Nei's genetic distance obtained three main clusters, River Kano, River Kano, and River Bagwai, with no outliers (Fig. 5). The configuration of the first clade revealed that the snails are more closely related. The present study indicated that the gene pool of *B. globosus* was microgeographically fragmented intraspecifically. Patterns of genetic differentiation at the fine-scale level in *B. globosus* are similar to other locations and species. For example, significant genetic homogeneity was previously reported for *Achatina achatina* (Etukudo et al., 2018), *Oreochromis niloticus* (Mahboob et al., 2019), and *Labeo ariza* (Ahammad et al., 2022), between geographic samples from different aquatic habitats.

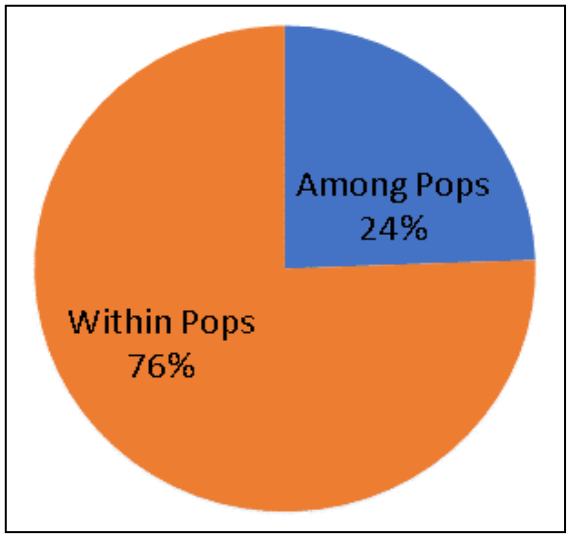

Figure 4: Percentages of molecular variance in *B. globosus* populations from three water bodies in Nigeria.

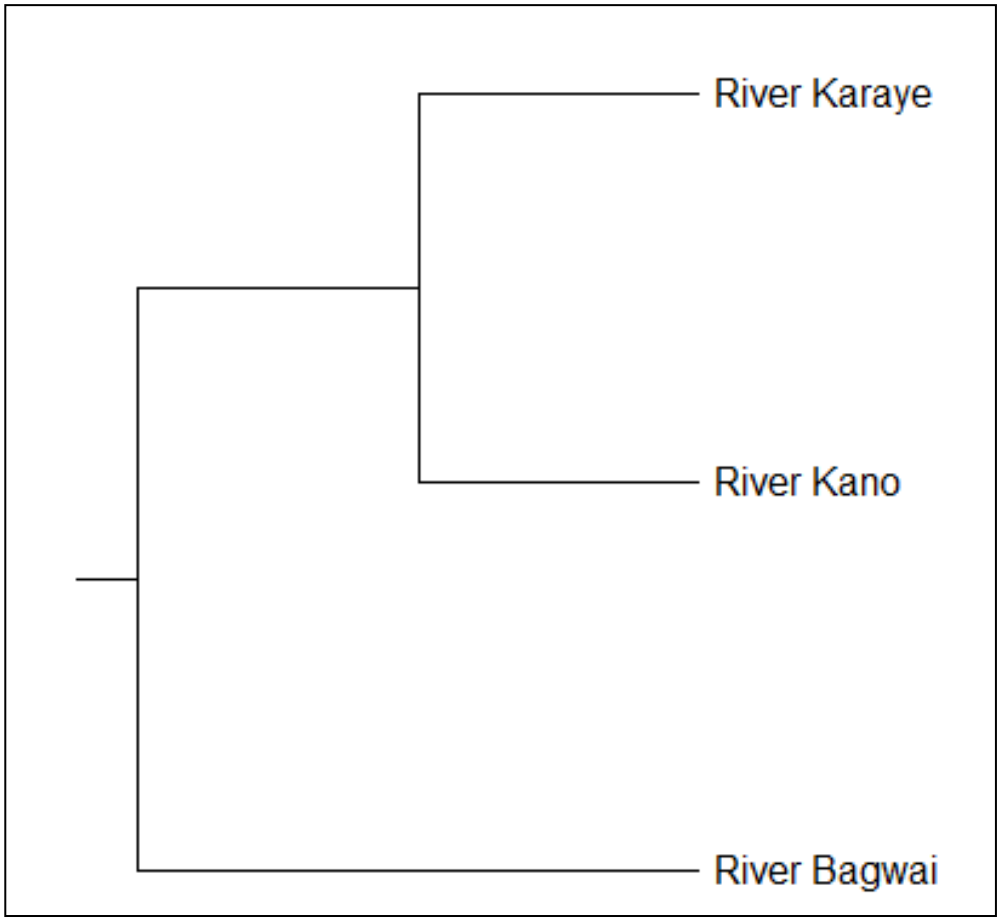

Figure 5: UPGMA Phylogeny constructed from Nei's genetic distance.

CONCLUSIONS

The present study revealed a wide variation of polymorphic loci among the three populations of *B. globosus*. The highest level of polymorphism (60.76%) was exhibited by the River Kano population, whereas the lowest level of polymorphism (55.70%) was exhibited by the River Karaye population. Restricted gene flow and high intraspecific population differentiation, but micro geographically fragmentation were observed. Moreover, RAPD confirmed the previous knowledge about its application as a quick and efficient method for generating DNA markers in aquatic organisms.

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