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GENETIC HETEROGENEITY OF PORTUNID CRAB POPULATIONS FROM THREE INTERCONNECTING TOPICAL LAGOONS

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Abstract

The degree of genetic variation in a population clearly specifies the structure of stocks and the probability of sustenance in future. The portunid crab, *Callinectes amnicola*, is presently managed as a single stock in Nigeria. Hence, genetic heterogeneity in this species from three interconnecting lagoons (Badagry, Lagos and Epe) was analysed using randomly amplified polymorphic DNA technique. The DNA yield and purity across populations ranged from 61.63 to 2983.34 ng/µl and from 1.68 to 1.86 respectively. The six RAPD primers: OPA-04, OPA-05, OPA-09, OPA-11, OPA-17 and OPAB-08 successfully amplified genomic DNA of 30 individual of *C. amnicola* from 3 populations with 1 region and 999 permutations. 86 RAPD fragments ranging from 96 to 1403 bp in length were generated. With 69 loci, the percentages of polymorphic bands for each primer across all populations were 60.87% (Badagry Lagoon crab), 62.32% (Lagos Harbour crab) and 66.67% (Epe Lagoon crab). Similarity index ranged from 0.848 to 0.893 and genetic distances from 0.114 to 0.165. The variations within and among the crab populations were 78% and 22% respectively. UPGMA Dendogram among *C. amnicola* populations using Nei's genetic distance obtained three main clusters, Epe Lagoon, Lagos Harbour and Badagry Lagoon, with seven outliers. The study established a relative geographical heterogeneity and limited gene flow across *C. amnicola* populations in coastal waters of Lagos, Nigeria.

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Introduction

The Lagoon crab, *Callinectes amnicola* belonging to the family Portunidae is a decapod crustacean of high commercial value in Nigeria (MORUF and LAWAL-ARE 2017). The species is generally cherished source of protein and minerals in human diet and animal feeds (CHINDAH et al. 2000, MORUF et al. 2019) and the most important food organism caught in the coastal (inshore) fishery and lagoons in West Africa (LAWAL-ARE and KUSEMIJU 2000). Portunid crab farming is well developed in Asia-Pacific region while supporting valuable commercial fisheries along the Atlantic coasts. These crabs inhabit a variety of aquatic habitats from the lower reaches of freshwater rivers, estuaries to coastal marine waters and are highly mobile, making it feasible for them to move between areas (LAWSON and OLOKO 2013).

Genetic status is essential information in fisheries management through stock enhancement or cultivation. The application of DNA markers has allowed rapid progress in aquaculture investigation of genetic variability and inbreeding, parentage assignment, species and strain identification, and the construction of high-resolution genetic linkage maps for aquaculture species (LIU and CORDES 2004). RAPD analysis uses a random oligonucleotide primer, obviating the need for knowledge of the sequences of the genome under investigation (KLINBUNGA et al. 2010); this will be useful particularly for a non-model species such as *C. amnicola* for which known nucleotide sequences of both coding and non-coding DNA in this species are rather limited. Randomly amplified polymorphic DNA (RAPD) analysis has been used to determine genetic diversity and identify useful genetic markers of various marine organisms (KLINBUNGA et al. 2007). FUJAYA et al. (2016) used RAPD markers to study the genetic variation of *Portunus pelagicus* from Makassar Straits, while SURESH and MADHURI et al. (2017) evaluated the genetic diversity of a mangrove crab of *Grapsus albolineatus*. KLINBUNGA et al. (2010) suggested that the RAPD technique is simpler and more cost-effective than amplified fragment length polymorphism (AFLP) analysis for monitoring levels of genetic diversity of *P. pelagicus*.

There is no data available regarding genetic diversity and population subdivisions of *C. amnicola* in Nigeria. This is the first report of genetic diversity of the species from Nigeria. The recognition of reproductively isolated and/or genetically differentiated populations within a species is of importance for broodstock selection and breeding programs (CONVER et al. 2006). The objectives of this study are determination of genetic diversity and intraspecific population differentiation of *C. amnicola* from three

interconnecting lagoons; Lagos, Epe and Badagry using RAPD analysis for which no data are available at present. Knowledge of the genetic diversity of *C. amnicola* in Nigeria waters is essential for the construction of an appropriate management scheme in this taxon. The basic information obtained can be applied to the construction of a genetic-based stock enhancement program for *C. amnicola*.

Materials and Methods

Study Site and Sample Collection

Three interconnecting lagoons (Badagry, Lagos and Epe) located in Southwest Nigeria were surveyed (Table 1, Figure 1). The Badagry Lagoon is part of a continuous system of lagoons and creeks along the Southwest Coast of Nigeria from the border with the Republic of Benin to the Niger Delta, with the depth of water ranging from 1–3 m and approximately 60 km long and 3 km wide (NDIMELE and KUMOLU-JOHSON 2012). The sheltered parts of sea areas where ships and boats can berth to offload and take on goods are regarded as habour. The 2 km wide Lagos harbour is geographically located at GPS co-ordinate of 6°39′16″N and 3°40′11″ E with average depth of 7.5 meters (LAWAL-ARE et al. 2018). Epe Lagoon has a surface area of 243 km^2, average depth of about 1.80 m and sandwiched between two other lagoons, the Lagos Lagoon (brackish water) to the west and Lekki Lagoon (freshwater) to the east (EDOKPAYI and IKHARO 2011).

Samples of live *Callinectes amnicola* were obtained from the catches of artisanal fishermen using traps in early hours of the day.

Table 1

Global Positioning System (GPS) coordinates of sampling locations

Locations	Latitude	Longitude
Badagry Lagoon	6°30′28″N	3°45′33″E
Lagos Harbour	6°39′16″N	3°40′11″E
Epe Lagoon	6°34′38″N	5°40′18″E

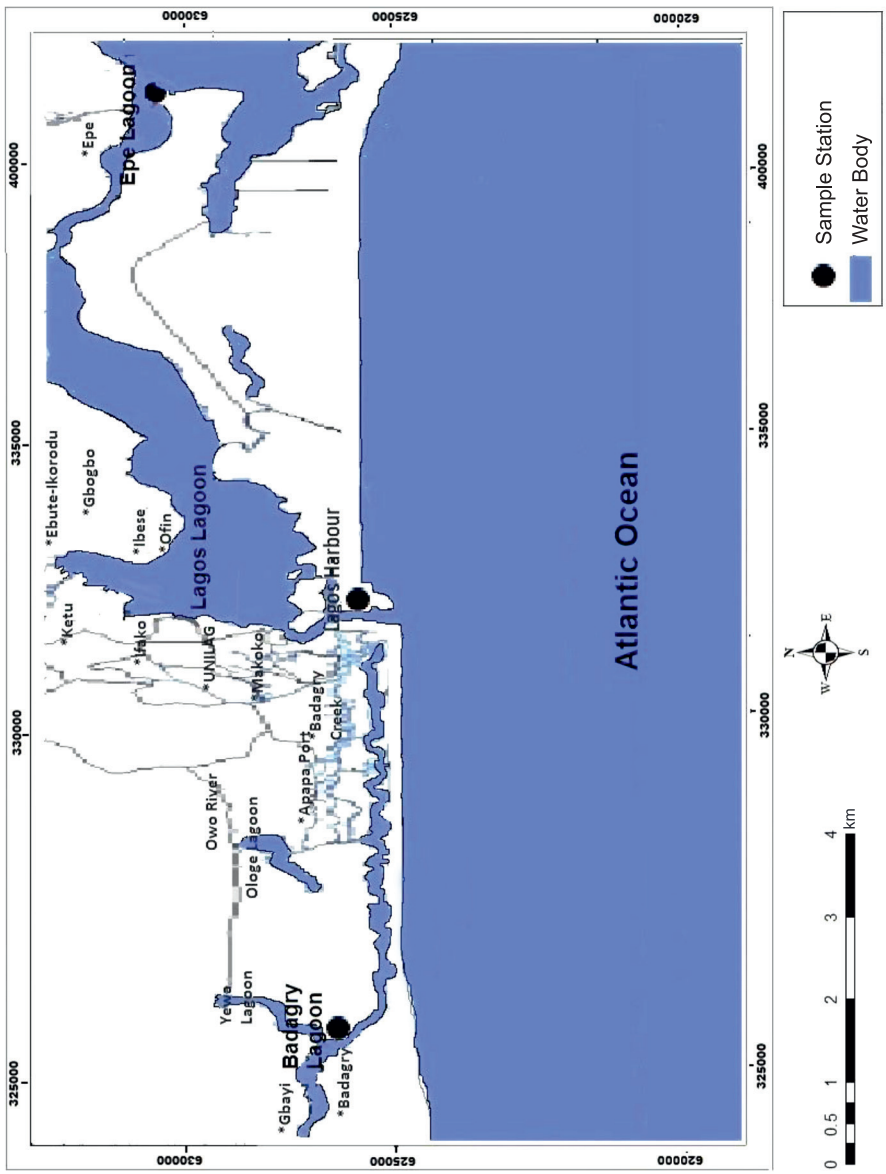

Fig. 1. Map of three interconnecting lagoons in Southwest Nigeria

Laboratory Protocol

Extraction of Genomic DNA

Genomic DNA was extracted from the muscle of the 1st periopod of each crab using a phenol-chloroform proteinase K method (KLINBUNGA et al. 1996). The concentration of the extracted DNA was spectrophotometrically estimated. DNA was stored at 4°C until needed.

Assessment of DNA Yield and Purity

DNA yield was determined with a nanodrop spectrophotometer (NANO 1000, China) based on maximum absorbance of DNA at 260 nm. 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.

RAPD-PCR amplification

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. Six RAPD primers: OPA-04 (5' AATCGGGCTG 3'), OPA-05 (5'-AGG GGT CTT G–3'), OPA-09 (5' GGGTAACGCC 3'), OPA-11 (5'-CAA TCG CCG T–3'), OPA-17 (5'-GAC CGC TTG T–3') and OPAB-08 (5' GTGACGTAGG 3') were used in the PCR reaction. 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 4 min, followed by 35 cycles of amplification (1 min denaturation at 94°C, 1 min annealing at 36°C and 1 min extension at 72°C). The process concluded with extension at 72°C for 10 min. analysis of the resultant amplification products was done at 100 V for 4 h 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 Electrophoresis

Agarose gel (1.5 gm/100ml) 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 loading buffer, they were electropho-

resed at 50 volts for 1 hour. Afterwards, agarose gel was stained with ethidium bromide (0.5 µg/ml) for 30 minutes and then photographed on U.V light with digital camera. RAPD-PCR technique can often produce non-reproducible amplification product (CALLEJAS and OCHANDO 2002).

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. This was done to maintain consistency across the samples of different populations. A band that occurred was noted as "1" while the absent band was marked as "0". Parallel comparison of the amplified products in the gel with standard molecular size marker (100 bp DNA ladder) gave an estimation of molecular sizes of the RAPD products. The program was fed with the resultant data to convert the polymorphic bands into dice distance. Dendrograms were thereafter produced by the unweight pair group method using arithmetic (UPGMA) average clustering. Finally, gel Images were used to analyze banding patterns.

Results and Discussion

A better understanding of population genetic structure is important to the effective fisheries management and conservation of genetic resources in exploited marine organism (BERT et al. 2007). The six RAPD primers successfully amplified genomic DNA of 30 individual of *C. amnicola* from 3 populations (Figure 2) with 1 region and 999 permutations. 86 RAPD fragments ranging from 96 to 1403 bp in length were generated. With 69 loci, the percentages of polymorphic bands for each primer across all population samples, 60.87% (Badagary Lagoon crabs), 62.32% (Lagos Harbour crabs) and 66.67% (Epe Lagoon crabs) suggested that inbreeding is not a major concern for this economically important species. The percentage of polymorphic bands in *C. amnicola* was greater than that of the Indian mangrove crab, where the level of polymorphic bands ranged from 24.6 to 60.1% in *Grapsus albolineatus* (SURESH and MADHURI et al. 2017). Although sample size from each geographic site in this study was limited, specimens were collected from different geographic locations in Lagos Coast of Nigeria. This should be sufficient to generate the preliminary data on genetic diversity and population differentiation of *C. amnicola* in Nigeria.

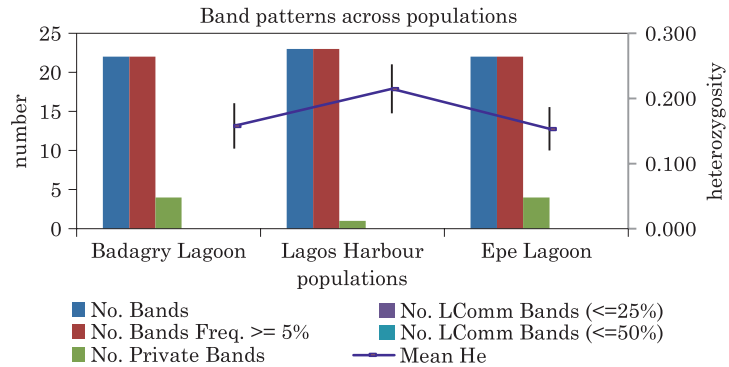

Fig. 2. Total band patterns for binary (diploid) data by populations

Theoretically, the extended planktonic larval stages of *C. amnicola* suggest high dispersal potential and the possibility of extensive gene flow between conspecific samples, at least on a geographic mesoscale of tens to hundreds of kilometers. Marine species with long larval phases are believed to have high levels of genetic variation within populations (FE´RAL 2002). According to Table 2, the large genetic distances among the geographic samples (0.114 to 0.165) indirectly reflected strong intraspecific genetic differentiation of *C. amnicola*. Generally, the levels of genetic distance between paired geographic samples did not reveal larger genetic distance with greater geographic distance (KLINBUNGA et al. 2010).

Table 2

Pairwise Population Nei Genetic Values of *C. amnicola* from three interconnecting topical lagoons in Nigeria

Population 1	Population 2	Nei Distance	Nei Identity
Badagry Lagoon	Lagos Harbour	0.140	0.870
Badagry Lagoon	Epe Lagoon	0.165	0.848
Lagos Harbour	Epe Lagoon	0.114	0.893

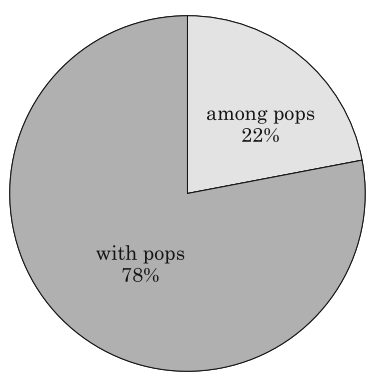

Fig. 3. Percentages of molecular variance in *Callinectes amnicola* populations from three interconnecting topical lagoons, Nigeria

The variations within and among the crab populations are 78% and 22% respectively (Figure 3) while the UPGMA Dendogram among *C. amnicola* populations using Nei's genetic distance obtained three main clusters, Epe Lagoon, Lagos Harbour and Badagry Lagoon, and seven outliers; samples 5 and 10 (Badagry Lagoon), 14 and 18 (Lagos Harbour), 21, 28 and 30 (Epe Lagoon) (Figure 4). The present study indicated that

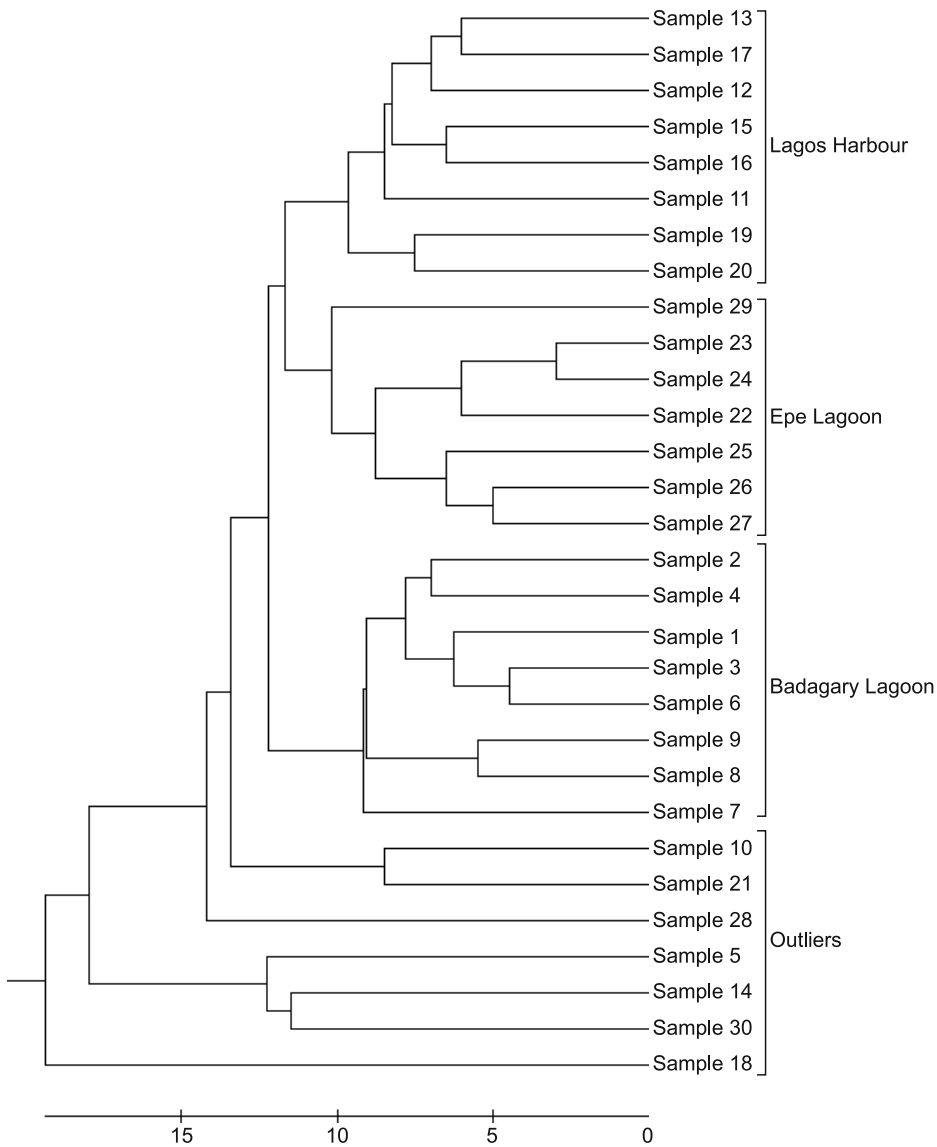

Fig. 4. UPGMA Dendrogram of three populations of *Callinectes amnicola* (De Rochecbrune 1883) using RAPD Technique

the gene pool of *C. amnicola* was not homogeneous but was microgeographically fragmented intraspecifically. Patterns of genetic differentiation at the fine-scale level in *C. amnicola* (e.g., between geographic samples approximately a few 100 km apart) were different from those of other lagoon species. For example, significant genetic heterogeneity was previously reported for the giant tiger shrimp (*P. monodon*; SUPUNGUL et al. 2000), the banana shrimp (*P. merguiensis*; HUALKASIN et al. 2003), and the abalone (*Haliotis asinina* and *H. ovina*; KLINBUNGA et al. 2003), between geographic samples from different coastal regions in Thai waters.

On the basis of the present study, three populations of *C. amnicola* were regarded as different genetic populations. From the management point of view, according to KLINBUNGA et al. (2010), these genetically isolated populations should be treated as separate management units. Stock enhancement to resolve consequent effects of overexploitation of natural *C. amnicola* may be carried out using a fine-scale level of local populations as the founders.

Conclusion

The relatively high degree of polymorphism in the population studied showed that the genetic diversity of *C. amnicola* in the Lagos coastal waters is high. High intraspecific population differentiation and restricted gene flow were observed. This information will be helpful formulating stock specific management for conservation of the species in Nigeria. In terms of aquaculture, domestication and subsequently selective breeding programs should be established for *C. amnicola* using the advantage of strong intraspecific genetic differentiation between geographically different samples of *C. amnicola* found in the present study. The proper source to be used as the founder stock for breeding programs of *C. amnicola* should be established from different genetic populations that are maintained separately. Interpopulational crosses may be carried out, possibly, to promote heterosis of economically important traits in this species. It is recommended that future studies should employ the use of newer and updated markers.

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