PRELIMINARY STUDY ON GENETIC VARIATION OF *Sudanonautes africanus* IN SOUTH-WEST, NIGERIA USING SDS-PAGE TECHNIQUE

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ABSTRACT
Freshwater crabs are a cheap and important source of animal protein for human beings. They also serve as bio-indicators of ecosystem disruption and also conservation icons. Despite their importance, there are still some unresolved issues in their phylogenies. This study is to observe the genetic variations in one of the species of freshwater crab, S. africanus from three different water bodies in South-west, Nigeria using Sodium Dodecyl Sulfate Polyacrylamide (SDS-PAGE) gel electrophoresis. Muscle protein was extracted from the individual crab in each of the population. The molecular weights of protein bands were observed and its ranges were 13.20 – 214.80kDa, 11.60 – 193.40kDa in Ogbese River and 19.30 – 193.40kDa in Owena River populations. UPGMA dendrogram formed to show the genetic similarities of the populations revealed two major clades. The study shows that there are intra and inter-population variations in each of the study locations.
Keywords: *West Africa, Nigeria, Shellfish, Freshwater crab, SDS-PAGE, genetic variation,*

INTRODUCTION

Shell fishes are nutritional sources of various minerals and high-quality proteins (Skonberg & Derkins, 2002). The majority of them are found in tropical waters where they occupy unique positions in the aquatic food chain. Crustaceans constitute important nutritional components in the diets of rural and urban communities (Fasakin, Bello-olusoji, & Oyekanmi., 2000). The problem of over-exploitation of fishery products by artisanal and industrial fisher folks, pollutants as well as incessant destructions of aquatic ecosystems (Dudgeon, 2002), have exposed these fishery products (shell and fin fishes) to genetic mutations and/or change in their phylogeny and morphological forms (Sodhi, Koh, Brook, & Ng, 2004, Sodhi *et al* 2008). These genetic changes have made it difficult to classify some of these aquatic organisms; hence this may have led to the evolvement of new species (Cumberlidge *et al.*, 2009).

Sudanonautes africanus is a common crab found in the eastern part of West Africa with a distributional range that includes the coastal rainforest regions of Nigeria, Cameroon, and Central Africa (Cumberlidge 1995c, 1999). They are found in large numbers in nearly all the available freshwater bodies in Nigeria, but they are underutilized except for local consumption (Bello–Olusoji *et al.*, 2010). They serve as a cheap and important source of protein for human beings. The crab meat is nutritionally valuable because of its richness in high-quality protein, minerals and vitamins (Adeyeye, 2002; Skonberg and Perkins, 2002; Omotayo et al., 2013) couple with low levels of fat and carbohydrates (Udo and Arazu, 2012). They also serve as bio-indicators of ecosystem disruption. Some are even used as conservation icons (Cumberlidge *et al*., 2006). According to Fagbuaro *et al*., 2013), they constitute a nuisance by damaging fishing nets in the water. Freshwater crabs are of medical importance as they serve as intermediate hosts of the human lung fluke, developing larvae of biting blackflies (*Simulium spp*) (Blair *et al.*,1998; Dai 1999; Rodriguez and Magalhaes, 2005). They are also vectors of the parasite *Onchocerca volvulus* (Crosskey, 1990) which causes river blindness in humans, Freshwater crabs are an integral component of the food webs in the aquatic ecosystems. They provide food for predators such as Otters, Mongooses, Herons, Kingfishers, and crocodiles among others (Butler and Marshall, 1996; Purves *et al*., 1994; Cumberlidge *et al*., 2009).

Electrophoresis (SDS-PAGE) has been used to study the relationship between fish species (Popoola *et al.,* 2014) as well as crustaceans. This technique is also used for the study of species genetic structure and the determination of phylogenetic relationships (Pineiro *et al.,* 2001). Sodium dodecyl sulphates Polyacrylamide gel electrophoresis (SDS-PAGE) involves the separation of proteins based on their size by heating the sample under denaturing and reducing conditions to unfold the proteins and coat it with SDS detergent to make it negatively charged.

The impacts of anthropogenic activities on the freshwater habitat as well as climatic changes have caused mutations, morphological, biochemical and molecular variations. These changes have made it difficult to classify and group these freshwater crabs (Cumberlidge *et al,* 2009). The status of freshwater crabs in South-west Nigeria has not been properly investigated**,** hence the cladistics and phylogenetics is not known. Therefore, the present study is aimed at estimating the level of genetic variability in terms of protein profile among the population of freshwater crabs.

Materials and Methods

Sample Collection

Geographically isolated populations of *S. africanus* were collected from three different River bodies of South-west, Nigeria, viz; River Ala (7º 263N, 5º 205E) located in Akure, the capital of Ondo State of Nigeria, River Ogbese (7º 261N, 5º 378E) located at Ogbese village (near Akure) and River Owena (7º 399N, 5º 896E) located along Akure-Ilesha road, Akure. Fifteen crab samples were collected from each population for SDS-PAGE analysis. The live specimens were hand-picked, pulled out of their holes or collected from farmers who caught them on their farmlands. The collected samples were transported to the Fisheries and Aquaculture Technology Laboratory, Department of Fisheries and Aquaculture Technology, Akure (FUTA) for preservation and further analyses.

Tissue Extraction

A piece of tissue (25mg) was collected from the chelae of the crab. The tissue was preserved in a plain sample bottle, filled with 2ml of 70% Ethanol and taken to the Biochemistry Department, Obafemi Awolowo University, Ile-Ife for SDS-PAGE analysis. The preserved chelae tissue was equilibrated with 0.5M Sodium Phosphate buffer (pH 7.0). The tissue was homogenized and centrifuged at 5, 000rpm for 20 minutes. The supernatant was collected and stored in a refrigerator. The tissue extracts were derivatized by weighing 0.1g of 2% SDS into forty-five Eppendorf tubes. To dissolve the solute, 250µl of each of the samples, 25µl of 2-mercaptoethanol (0.5M), 15µl of tris-buffer, 25µl of bromophenol blue and 200µl of glycerol was added to the solute. The mixture was shaken vigorously and boiled in a water bath for 3 minutes at 90ºC. After boiling, the protein samples were allowed to cool before they were loaded on the wells of the electrophoretic gel.

Electrophoresis

The methodology for gel preparation and casting is as described by Laaemmli, (1970). After electrophoresis, the gel was fixed in a fixing solution and destained to reveal the position of the protein bands in the gel and comparing with the standard of known molecular weights.

Statistical analysis

Photographs of the gels were captured and the positions of the protein molecular markers were scored for the presence and/or absence of protein bands. Dendrograms and similarity matrices were obtained by the Unweighted Pair Group Method (UPGMA) by using Past software.

Results

A total of 296 bands were observed from the three populations (Tables 1-3). The highest number of bands (153) was found in Ogbese population, followed by 77 bands in Owena River and 66 bands in population from Ala River.

In Ala population, the samples have a maximum of eight bands (in sample 11). The molecular weight ranges from 13.20kDa to 214.80kDa (Table 1).

In Ogbese River, in terms of their protein bands, the highest number of band was seen in sample 29 with 23bands. Samples 20 and 21 have a similar number of related bands. The molecular weight ranges from 11.60kDa to 193.40kDa (Table 2). Samples 17, 24 – 29 have protein bands with the same molecular weight. In Owena River, Samples 36, 37, 38 and 39 have similar protein bands (2 protein bands). The molecular weight of the population from Owena River ranges from 22kDa to 194kDa (Table 3). A high molecular weight band was seen in the sample from Ala River (with 214kDa) while the population from Ogbese river has the largest protein profile (Sample 29) with 23 bands.

Table 1: Molecular weights (kDa) of Proteins in *S. africanus* Proteins from Ala River

Samples/ Bands	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
A	193.00	183.00	121.00	121.00	125.00	121.40	125.00	142.30	201.60	195.40	214.80	-	-	-	-
B	13.20	120.00	15.50	14.53	12.40	20.00	21.25	107.00	142.30	142.30	142.30	-	-	-	-
C	-	14.52	13.20	13.21	-	13.20	13.20	21.30	64.40	64.40	114.00	-	-	-	-
D	-	-	-	-	-	-	-	13.20	21.30	30.12	91.30	-	-	-	-
E	-	-	-	-	-	-	-	-	13.64	23.40	67.00	-	-	-	-
F	-	-	-	-	-	-	-	-	-	13.64	30.1	-	-	-	-
G	-	-	-	-	-	-	-	-	-	-	23.78	-	-	-	-
H	-	-	-	-	-	-	-	-	-	-	13.64	-	-	-	-

Where letters A- H are the different bands on the Electrophoretic gel

Table 2: Molecular weights (kDa) of Proteins in *S. africanus* Proteins from Ogbese River

Samples/ Bands	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
A	74.02	81.60	89.40	19.00	102.50	102.5	19	102.5	22.2	22.2	22.2	193.4	173.3	168.6	87.3
B	50.10	69.40	36.20	-	19.00	19.	-	76.5	-	-	-	87.3	85	76.12	74.1
C	36.20	46.90	29.40	-	-	-	-	17.1	-	-	-	76.1	70.1	64.6	33.4
D	24.50	28.80	19.00	-	-	-	-	11.6	-	-	-	22.2	50.5	46.5	-
E	17.70	26.10	-	-	-	-	-	-	-	-	-	-	30.8	30.8	-
F	-	23.70	-	-	-	-	-	-	-	-	-	-	22.2	22.2	-
G	-	22.20	-	-	-	-	-	-	-	-	-	-	-	-	-

Where letters A- G are the different bands on the Electrophoretic gel

Table 3: Molecular weights (kDa) of Proteins in *S. africanus* Proteins from Owena River

Samples/ Bands	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45
A	85	87.3	193.4	194	191.5	191.5	191.5	176.2	90.4	22.5	21.9	21.9	21.9	21.9	110
B	70	76.1	92.2	139.2	88	104	110	88	29.7	-	-	-	-	-	88
C	-	35.3	76.1	92.2	72.4	88	90.4	50.4	22.5	-	-	-	-	-	72.4
D	-	23.4	53.3	76.12	50.4	50.4	76.5	39.3	-	-	-	-	-	-	59.6
E	-	19.3	44	53.3	41.5	34.2	59.6	34.2	-	-	-	-	-	-	50.4
F	-	-	35.3	44	34.2	31.4	50.4	29.7	-	-	-	-	-	-	41.5
G	-	-	29.2	37.3	31.4	21.9	41.5	27.4	-	-	-	-	-	-	36.1
H	-	-	24.7	33.4	27.4	-	36.1	21.9	-	-	-	-	-	-	25.2
I	-	-	-	24.7	22	-	31.4	-	-	-	-	-	-	-	21.9
J	-	-	-	-	-	-	27.4	-	-	-	-	-	-	-	33.2
K	-	-	-	-	-	-	22.5	-	-	-	-	-	-	-	29

Where letters A- K are the different bands on the Electrophoretic gel.

The protein gel electrophoresis bands of *S. africanus* from the three populations (Plates 1-5) show variations in terms of the number, position, and intensity of the stains. From the gels, the number of bands produced varies from one to twenty-three and their molecular weights vary between 13.20kDa to 214.80kDa. It was also observed from the protein profile that there was an overlapping in the molecular weights of some bands (13.20, 19.00, 21.90 and 22.20kDa). Band with 214.80 is only peculiar to lane 11.

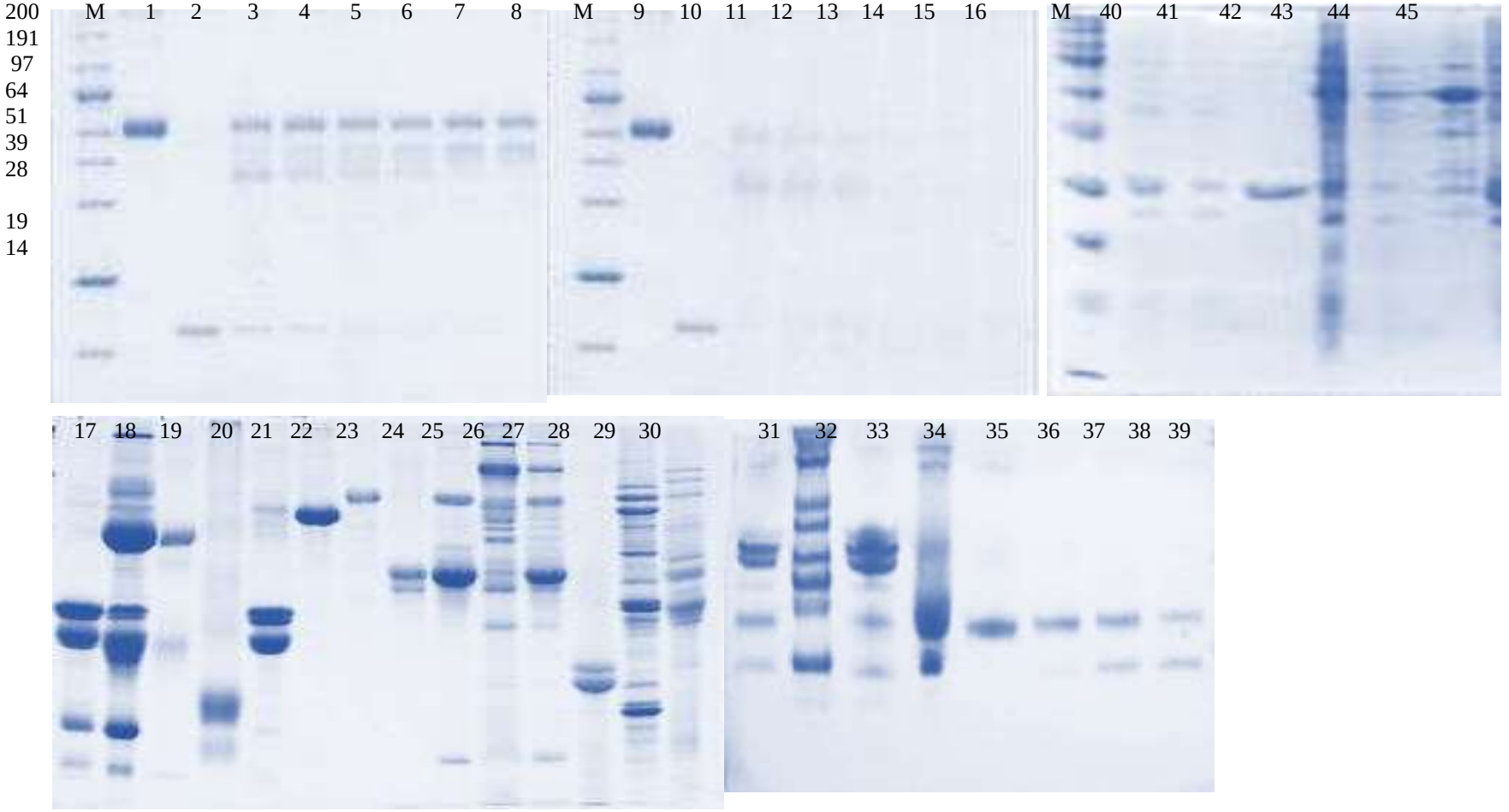

Figure 1. Protein profiling of muscle tissue of *S. africanus from* three populations (Ala; 1-15, Ogbese;16-30 and Owena;31-45) and M-marker

A dendrogram (Fig.1) was generated using the similarity coefficient based on the molecular weights (kDa) of the soluble chelae tissue proteins extracted from the crab samples. The clusters obtained from the dendrogram revealed that the *S. africanus* collected from three populations were grouped into two clades.

Clade 1 contains two samples, 37 and 45. These samples exhibit similar molecular weights (protein profile) and have more bands than the other samples in the three study locations. Cluster 2 has two sub-clusters (Cluster 2A and 2B). Cluster 2A has two groups namely A1 and A2. Group A1 has two sub-groups (A11 and A12). Group A11 has only sample 17 on one clade (A111) while samples 28, 29 (from Ogbese River) and 36 (from Owena River) were found on clade A112. The second stem on A1 (A12) has samples 33, 35, 36 and 38 (from Owena River). While stem A2 exhibits inter-population relatedness with Samples 34 (From Owena River) and 11 (from Ala River) found on it. This implies that although the samples from the same sub-group have similar molecular weights, they are slightly different from members of the other sub-groups.

Group B also has two sub-groups, B1 and B2. B1 has two stems (B11 and B12) where the first stem has samples 16 (from Ogbese River) and 32 (from Owena River). The second stem (B12) possesses 27 samples on it. On the stem, samples 3, 4, 6 and 7 (from Ala River) shared similar muscle protein profiles. Also on the same node, samples 12 – 15 (from Ala River) exhibit the same protein profile structure, samples 20 and 21 exhibits the same protein profile structure. Similarly, samples 19, 22, 24, 25, 26, 40 – 44 also exhibit the same protein profile structure, hence the reason for the unbranched node on the stem. The second sub-group B2 has two nodes where samples 9 and 10 show similarities in their protein profile, samples 2 and 8 (from Ala River) also share the same muscle tissue protein profile whereas, sample 27 (from Ogbese River) show a slight variation in the muscle tissue protein profile on the same node.

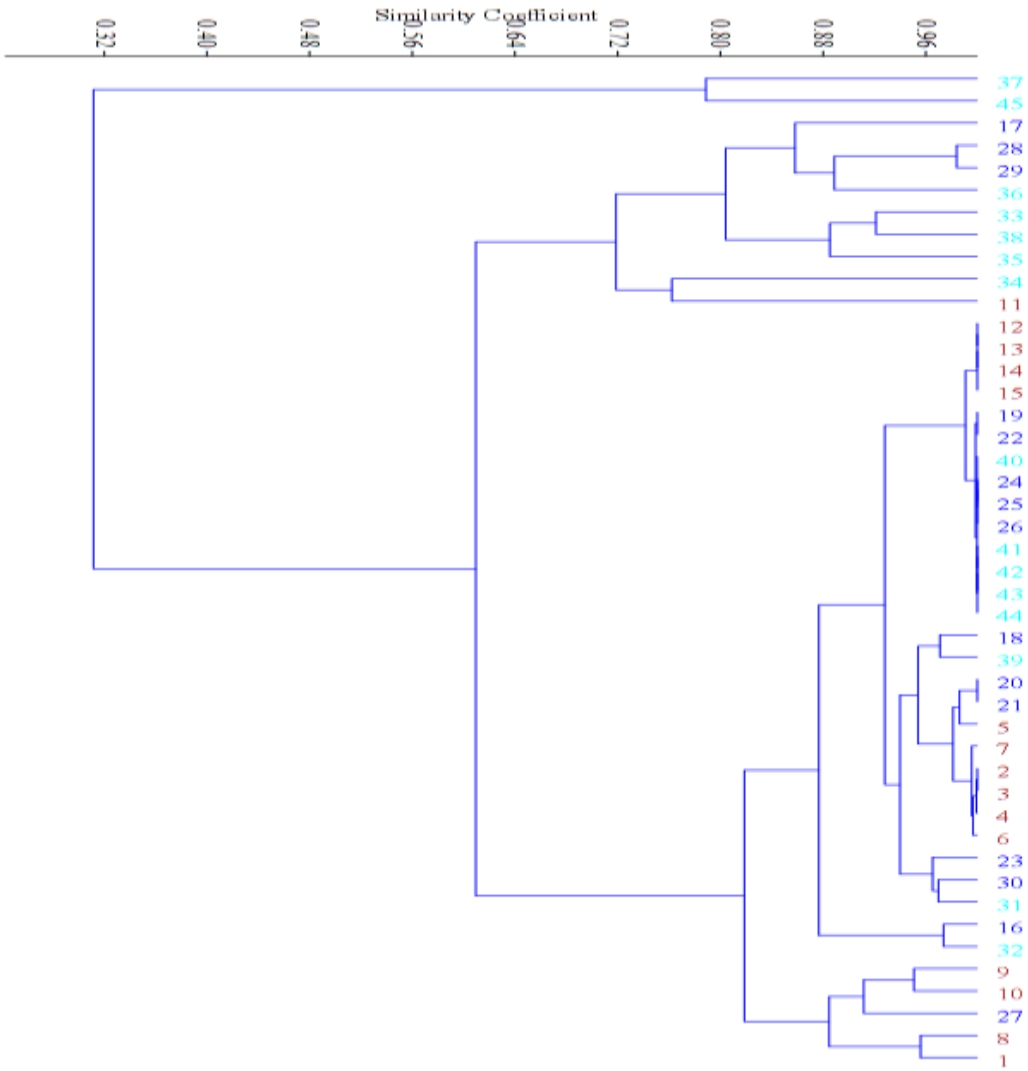

Fig.1: Dendrogram generated based on the molecular weight (kDa) of proteins of *S.africanus* in the three study locations.

Discussion

One of the aims of assessing stocks of the crab population is to make inferences about the historical processes affecting relationships (phylogenetics) among groups and geographical distributions (phylogeography) of an organism. It is a powerful tool to understand an organism's evolutionary history (Chu *et al.,* 2002).

Although the freshwater crabs rank among the most important invertebrates in freshwater habitats across Africa, their taxonomic representations were not well known until recently. However, the impression is changing due to an increased interest in the identification, taxonomy (classification), phylogeny, diversity, distribution pattern and conservation status of freshwater crabs (Cumberlidge & Daniels, 2007; Cumberlidge, Von Sternberg, & Daniels, 2008; Cumberlidge *et al.*, 2009; Daniels, Cumberlidge, Pe´rez-Losada, Marijnissen, & Crandall, 2006).

Protein electrophoresis is a technique used in the determination of genetic structures of an organism and the determination of phylogenetic relationships of similar or different organisms (Valemela *et al.,* 2017). Since Proteins are gene products, the electrophoretic movements of different proteins in closely related species or different populations can be genetically interpreted. Haniffa *et al.,* (2017) used different electrophoretic techniques to identify the differences among fish species and muscle proteins. In this present study, the SDS-PAGE assay has successfully generated the muscle tissue protein profile of *S. africanus* in the three study locations. The study reveals some degree of variations in the chelae tissue protein profiling of the studied species of crab. Similar values in the molecular weights indicate that some proteins are common to the crab samples in the study locations. The presence of some unique molecular weights of proteins in the samples is an indication that certain proteins are peculiar to these samples, hence such samples do not vary from one another. The chelae tissue protein profile observed through SDS-PAGE showed variations in *S. africanus* in the three study locations.

The presence of some proteins with similar molecular weights in *S. africanus* in the three study locations confirms that they have a close ancestral relationship. Omoniyi and Fagade (1998) reported similar observations in the muscle protein electrophoretic studies of *Oreochromis niloticus* and *Sarotherodon galileus;* Das *et. al.,* (2005) also revealed the genetic similarity between strain and reference strain (MTCC 646) in protein fingerprinting of *A. hydrophilia*. The result of SDS-PAGE analysis is also in consonance with the works of Wei and Musa (2008) on the whole-cell protein profiling of African catfish, Red Hybrid tilapia, Asian swamp eel, and Snakeskin gourami. The molecular weights of the population in Ala River is similar to the study of Valemela *et al.,* (2017) on *L. argentimaculus*, *L. fulviflamma*, *L. fulvus*, *L. lemniscatus*, *L. lutjanus* and *L. madras*.

According to Sudhakar *et al.,* (2012), the biochemical constituents in animals vary with season, size of the animal, stage of maturity, temperature, and availability of food, e.t.c. UPGMA dendrogram revealed the genetic distances and variations in tissue protein profile of *S. africanus* in the three study locations. The result showed that there are wide variations in terms of their protein profiles. These variations may be attributed to the different anthropogenic activities carried out in the study areas. Secondly, the time of collection of the crab (dry season) may have altered the protein profile of some of the crabs. Thirdly, the method of preservation of the muscle tissues may have also caused these wide variations in the protein profile. Lastly, seasonal migrations may have caused some genetic mutations to occur, hence the biochemical variations in *S. africanus* as revealed by the present study. Results from the muscle tissue of *S. africanus* are important for biomarker identification among the crab population.

Conclusion

The similar molecular weights of the protein bands from the SDS-PAGE indicate that the crabs in the three study locations have a close ancestral relationship. The dendrogram of the molecular weights of the crab population from the three study locations revealed that the species of crab varies genetically to each other with respect to their sample locations. Therefore, the study revealed that *S. africanus* in South-west Nigeria varies genetically which means they might be of the different strains.

Recommendation

This study may serve as the basis in the proper phylogenetic of fresh-water crabs. It may also serve as a baseline for protein and proteomic studies in fresh-water crabs.

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