Journal of
Fisheries and Aquatic Science

ISSN 1816-4927

Academic
Journals Inc.

www.academicjournals.com

OPEN ACCESS Journal of Fisheries and Aquatic Science

ISSN 1816-4927
DOI: 10.3923/jfas.2023.10.19

Research Article
Genetic Similarity Levels in Nile Tilapia of Some Nigerian Water Bodies Using SSR Markers

[1]Mojekwu, T.O. and [2]C.I. Anumudu

[1]Department of Biotechnology, Nigeria Institute of Oceanography and Marine Research, PMB 12729, Lagos, Nigeria
[2]Department of Zoology, University of Ibadan, Ibadan, Oyo, Nigeria

Abstract

Background and Objective: In Nigeria, there is a paucity of information on the genetic similarity among wild *Oreochromis niloticus* species. Though there are assertions based on morphological observation, by fish farmers and some researchers of fisheries that most of these species in the wild are no longer pure and possibly hybridizing. Most molecular assessments are usually among tilapia species with limited sample coverage of *O. niloticus* species or of a lower marker. This was a preliminary investigation of the genetic similarity of *Oreochromis niloticus* from selected water bodies along the River Niger, in six states of Nigeria, using SSR markers. **Materials and Methods:** Similarity levels among *Oreochromis niloticus* from water bodies in six states in Nigeria were assessed using seven SSR markers with their various primers (UNH 208, 143, 104, 022, 119, 180 and 156), which were selected from the NCBI database. Only UNH 104, UNH143 and UNH 022 produced DNA amplifications. Phylogenetic representation using UPGMA cluster analysis produced a dendrogram with a total of 118 clusters grouped into 7 major clusters with up to 8 subgroups, indicating different degrees of variation and similarities. **Results:** Genetic similarities were observed at various coefficient levels, possibly due to hybridization. At the coefficient level of 1 or 100%, 39 clusters occurred with different samples on each cluster indicating they are genetically the same. Samples from South Delta (SSD), South West Oyo (SWO) and North West Kebbi (NWK) states were always present in each cluster at this coefficient level. A lot of migration is likely taking place among these fishes. The seven major clusters A, B, C, D, E, F and G at varying degrees of similarity 73, 67, 64, 59, 56, 48 and 44% showed the decreasing order of genetic similarity. **Conclusion:** These data supported the view that the very high similarity between tilapia from these water bodies stems from its highly prolific nature which may have led to a high probability of hybridization between species and strains. The genetic distance between strains may suggest that there are varying levels of genetic variability among *O. niloticus* in Nigeria water bodies under study.

Key words: Genetic similarity, *O. niloticus*, SSR, water bodies, Nigeria

Citation: Mojekwu, T.O. and C.I. Anumudu, 2023. Genetic similarity levels in Nile tilapia of some Nigerian water bodies using SSR markers. J. Fish. Aquat. Sci., 18: 10-19.

Corresponding Author: Mojekwu, T.O., Department of Biotechnology, Nigeria Institute of Oceanography and Marine Research, PMB 12729, Lagos, Nigeria

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

There are over 75 species of tilapias in the world, out of which about nine species are used in aquaculture globally. There has been a moderate increase in the global Nile tilapia (*Oreochromis niloticus*) production, accounting for 8.3% of global aquaculture finfish production in 2018[1]. Hence, it significantly increases world tilapia production from freshwater aquaculture. In Nigeria many tilapia species abound in the wild and some are cultured, but *Oreochromis niloticus* appears to be one of the more popular among fish farmers[2], possibly due to its economic profitability in semi-flow-through culture system[3]. Genetic characterization of this species in Nigeria water is very important for determining variability and similarities among populations. This will also be useful in aquaculture and fisheries stock management.

Molecular genetic markers are strong tools in determining the genetic uniqueness of individuals, populations or species[4-6].

Simple sequence repeats (SSRs) otherwise called Microsatellites have been applied as a preferred marker in most genetic mapping and genome analysis. This is because they are ample and well spread in the genomes of eukaryotes[7-9]. The SSR- PCR is a useful tool for estimating the degree of genetic similarity among fish species[10-13]. They have been applied in differentiating different tilapia species[14,15]. Microsatellite markers have been utilized to assess red tilapia stock[16] and to determine the genetic diversity of genetically improved farm tilapia[17,18].

Many tilapia microsatellites have been used to study the inheritance of traits[19-22]. Salt tolerant tilapia, growth and sex-determining loci of *Oreochromis mossambicus*× *Oreochromis* spp., has been mapped using microsatellite[11,23]. Disease resistance tilapia has also been developed through selective breeding using microsatellite markers[24]. Microsatellite markers associated with cold tolerance in *Oreochromis niloticus* have been screened and identified[25].

Nonetheless, primer development is quite stressful and usually expensive[26]. Another disadvantage is that some alleles do not amplify in PCR reactions[27]. Therefore, this research was a preliminary investigation of the genetic similarity of *O. niloticus* in selected water bodies along the river Niger in six states in Nigeria using SSR markers.

MATERIALS AND METHODS

Study area and fish samples: Two hundred and four specimens identified as *O. niloticus* species were obtained from six states representing the geo-political zones in Nigeria (Fig. 1). The collection was done between January, 2013 and October, 2013 with the help of local fishermen. Fish were kept on ice until transferred to the lab and frozen at -80°C until analyzed.

Thirty-four individuals from two water bodies in each of the states were sampled: Adamawa (Gariyo and Kiri Lakes), Kebbi State (Birnin Kebbi/Rimer River), Kogi State (Lokoja confluence and Sarkin Noma River), Oyo State (Eleyele lake and Oba Dam), Anambra State (Igbariam and Otuocha Rivers) and Delta State (Warri- Umuochi/Ona River). The laboratory analysis was conducted at the Biotechnology Laboratory of the Nigerian Institute for Oceanography and Marine Research, Victoria Island, Lagos Nigeria.

DNA extraction and SSR-PCR: Genomic DNA was isolated using the salting out method from the caudal fin according to Lopera-Barrero *et al.*[28]. Caudal fins (approximately 3.5-4.5 g) were placed in Eppendorf microtubes with 90% absolute ethanol and maintained in a freezer at -20°C. Lysis buffer (550 µL) containing 7 µL of 200 µg mL^{-1} of proteinase K was added. The samples were incubated in a water bath at 50°C for 12 hrs. Sodium chloride (660 µL of 5 M) was added and centrifuged at 12,000 rpm for 10 mins. The supernatant was transferred to a new tube and 700 µL of cold absolute ethanol was used to precipitate the DNA. The samples were incubated at -20°C for 2 hrs. The DNA sample was centrifuged and washed with 700 µL of 70% v/v ethanol. The sample was re-suspended in 80 µL TE buffer (10 mM of Tris pH 8.0 and 1 mM of EDTA). Ribonuclease (30 µg mL^{-1}) was added and incubated at 42°C for 4 hrs. This was stored in the freezer at -20°C before PCR. The concentration and purity of the extracted DNA were determined by measuring absorbance at 260 and 280 nm with a Biorad Nano Spectrophotometer.

The PCR reactions were performed in a Biorad I-cycler with the following program. Initial denaturation step at 95°C for 5 min, 35 cycles of 30 sec at annealing temperature (45-60°C) and 1 min at 72°C, followed by a final elongation step of 10 min at 72°C. The amplified DNA fragments were electrophoresed using 1.5% (w/v) agarose gel and stained with ethidium bromide. The amplified products were visualized on a UV transilluminator and photographed. The SSR primers used were selected from the database at the National Center for Biotechnology Information (Table 1)[29].

Phylogenetic analysis: Phylogenetic analysis was done using alleles count, the alleles were scored and the distance (UPGMA) method was used to construct a dendrogram that showed the similarities and differences between the genes. The analysis was carried out using NYSYS software.

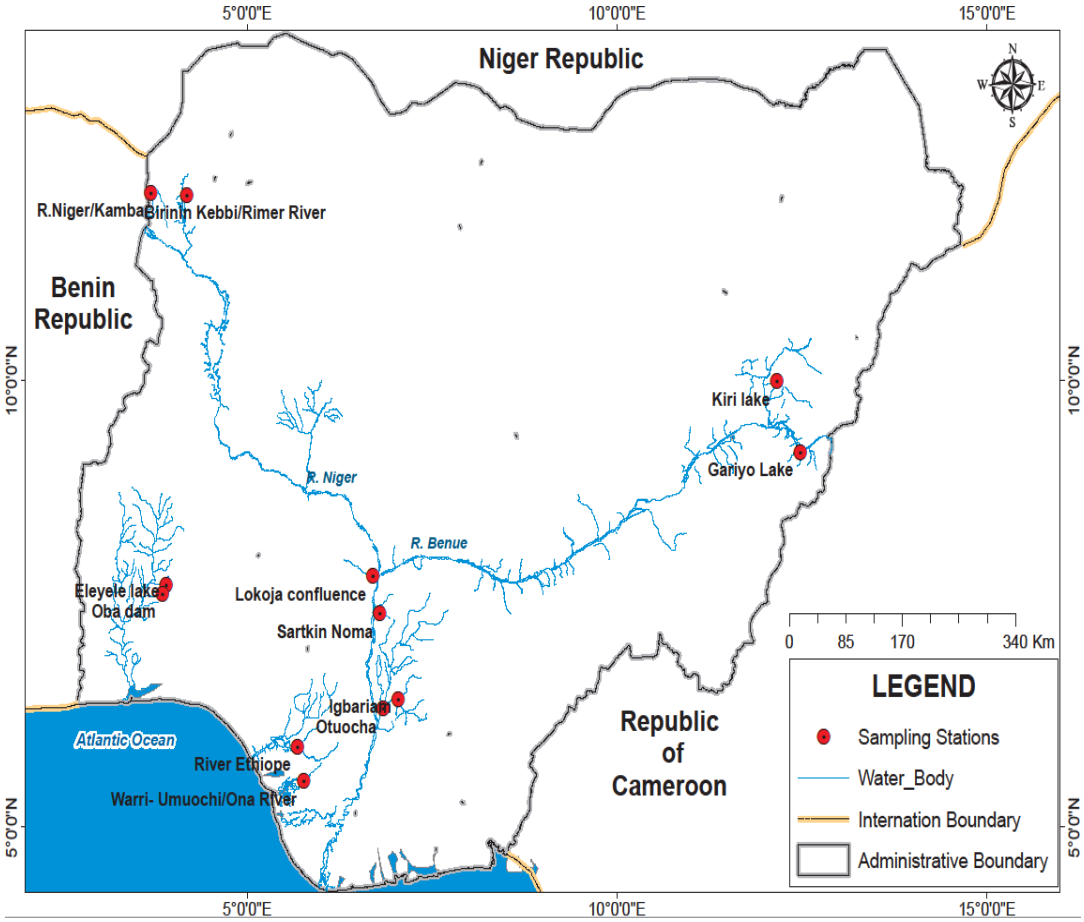

Fig. 1: Map of Nigeria showing sampling sites

Table 1: Microsatellite markers and primers used in this study from Kocher *et al.*[29]

Marker	Expected band size	Primer (5'-3')	Gene Bank accession
UNH208	100	F CTTCTTGGCCTACAATTT	G12359
		R CAGATGGGTGATAGCAA	
UNH143	213	F GCATGCGTGGGTGTTTGTATTGTG	G12295
		R TTCGCTCTTTTGTGGGGTGTCTGA	
UNH104	138	F GCAGTTATTTGTGGTCACTA	G12257
		R GGTATATGTCTAACTGAAATCC	
UNH022	257	F GCAGCTCTCGCTCTCTCTCT	G36423
		R CGTGCTGAGTTGTGGAGGTA	
UNH119	158	F GCAGATATACCTGAGAAAAC	G12272
		R AGGTGATAACAAAAAGGGAAT	
UNH180	155	F GCAACTAATCACACAATTTT	G12332
		R GTTTAAGTTAAAAACAAATTCGTTT	
UNH156	188	F GATCATTGGGTGTCATTACTCTCA	G12308
		R TCTCTGCCTTCCTCCATTACTCA	

F: Forward primer (5'-3') and R: Reverse primer (3'-5')

RESULTS AND DISCUSSION

Genetic variation in any species is a resource that is manifested at two different hierarchical levels. The first level is explicit and includes the genetic differences between individuals within a population. The second level of genetic variation is that which exists among the various populations[30-32]. The SSR markers have become one of the most popular genetic markers because of their high[9] polymorphism and PCR-based analysis[10,15]. Microsatellite bands in this study were always unstable (i.e., strong, faint, fuzzy and sharp) bands generated with each primer because one or more copies of DNA may exist per genome or may be attributed to the varying annealing

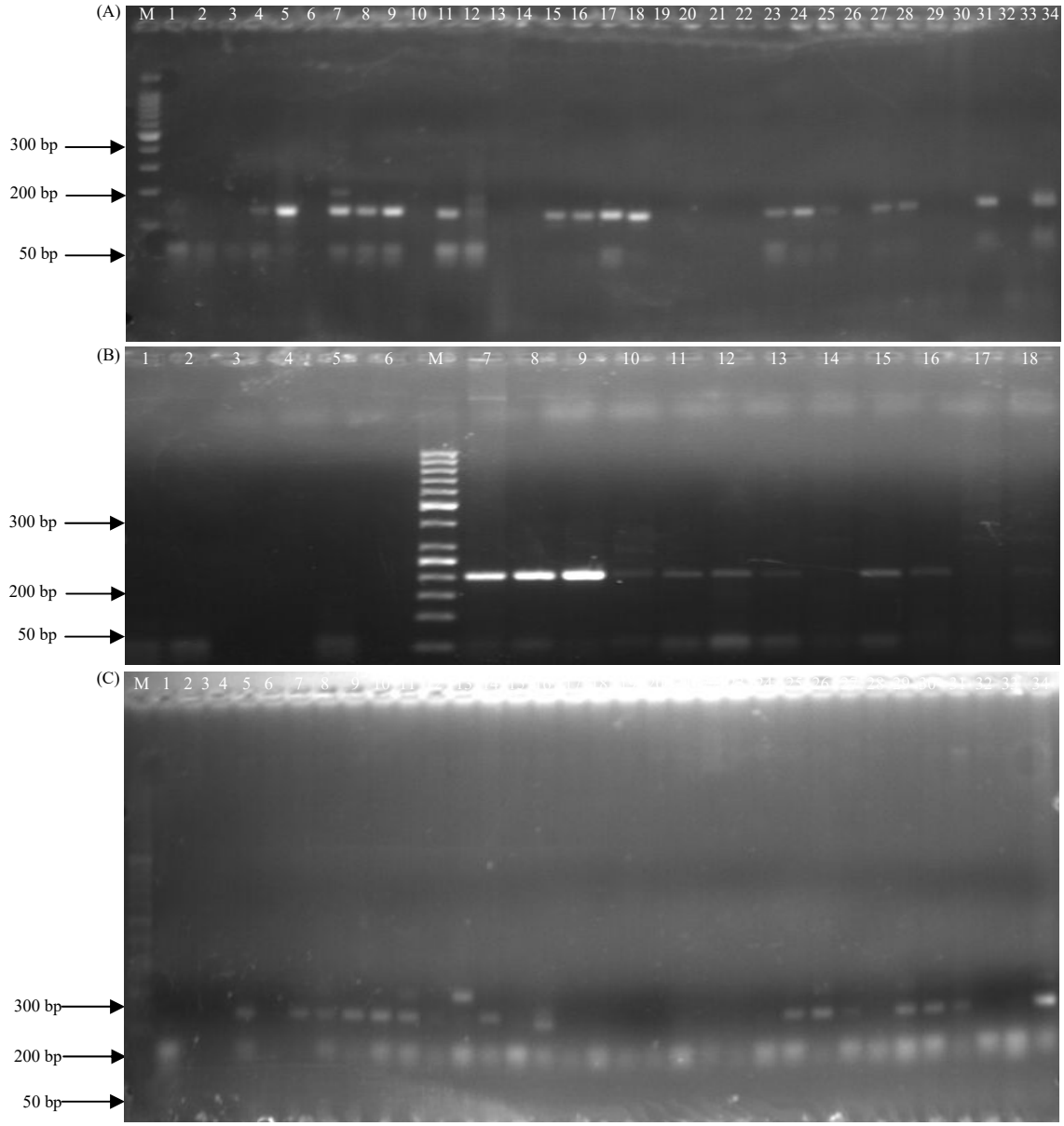

Plate 1: Amplified SSR loci for individuals from North East, Nigeria (Adamawa State)

Lane M is 1 kb DNA ladder, A, B, C are primers UNH 104, UNH143 and UNH 022 showing fragments sizes 138, 200 and 257 bp, respectively and NB: Electrophoresis of remaining 16 individuals for b was run on a different plate not shown here

process between the primer and the DNA. This problem of mixed bands shows the well-known sensitivity of PCRs[10]. The SSR fragments generated by primers UNH 208,119,180 and 156 showed low polymorphism among the fishes studied. These primer sequences may have annealed to variable sequences, which might be of great utility at lower taxonomic levels, e.g., for the differentiation of closely related species. However, microsatellite fragments generated by three primers UNH 143, 104 and 022 showed both stable amplification and a high degree of polymorphism. The DNA fragment sizes of these primers range from 138, 200 and 257 bp (Plate 1), respectively. Their sequences may be considered as more conserved sequences, which are most useful in higher taxonomic levels and evolutionary relationships. Their high polymorphism and PCR-based analysis have made them one of the most popular genetic markers[10,15]. Some microsatellite loci have very high numbers of alleles per locus (>20), making them very useful for applications such as parent-offspring identification in mixed populations, while others have lower numbers of alleles and may be more suited for population genetics and phylogeny[11,15,19,33].

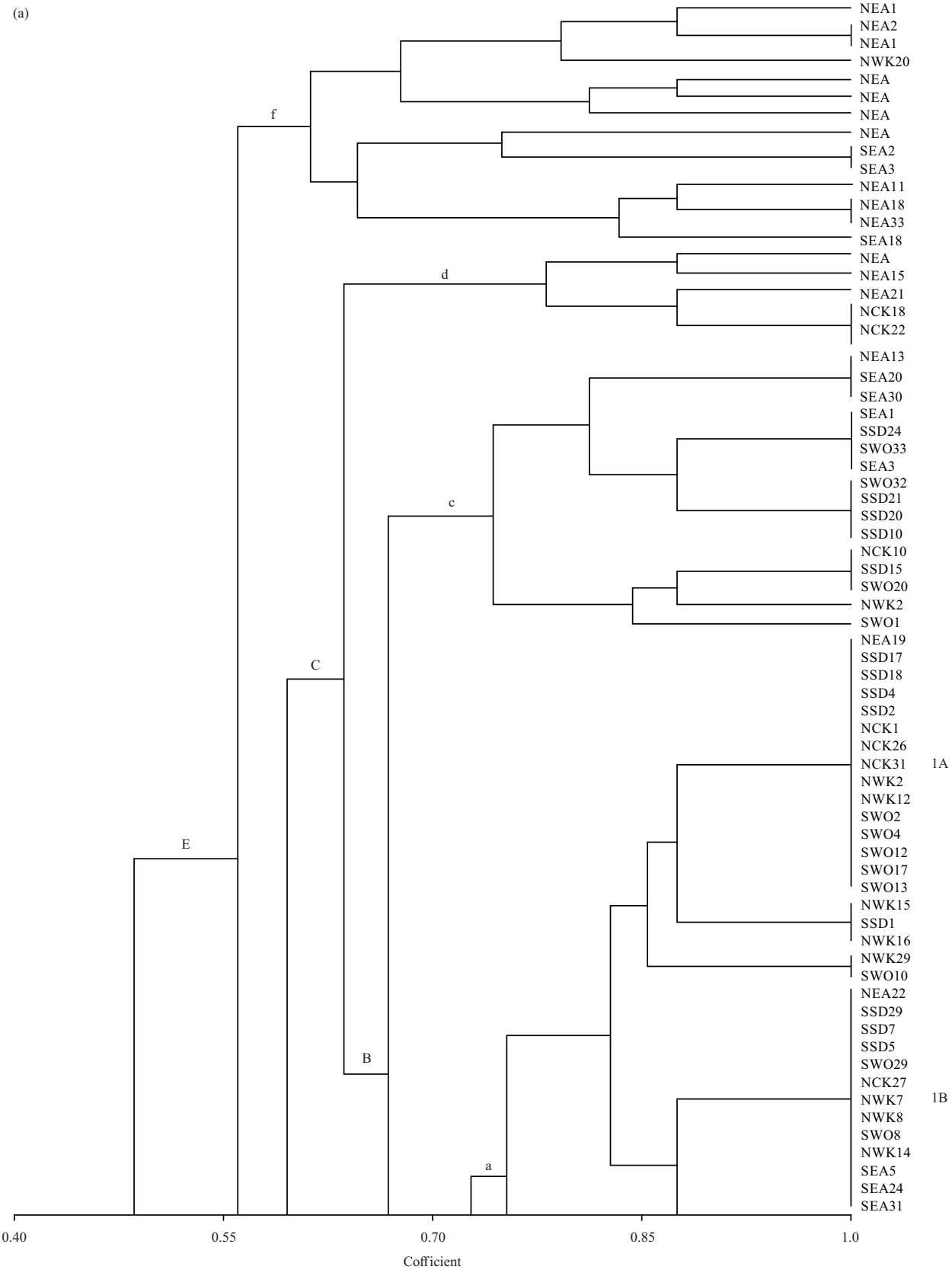

Fig. 2(a-c): Continue

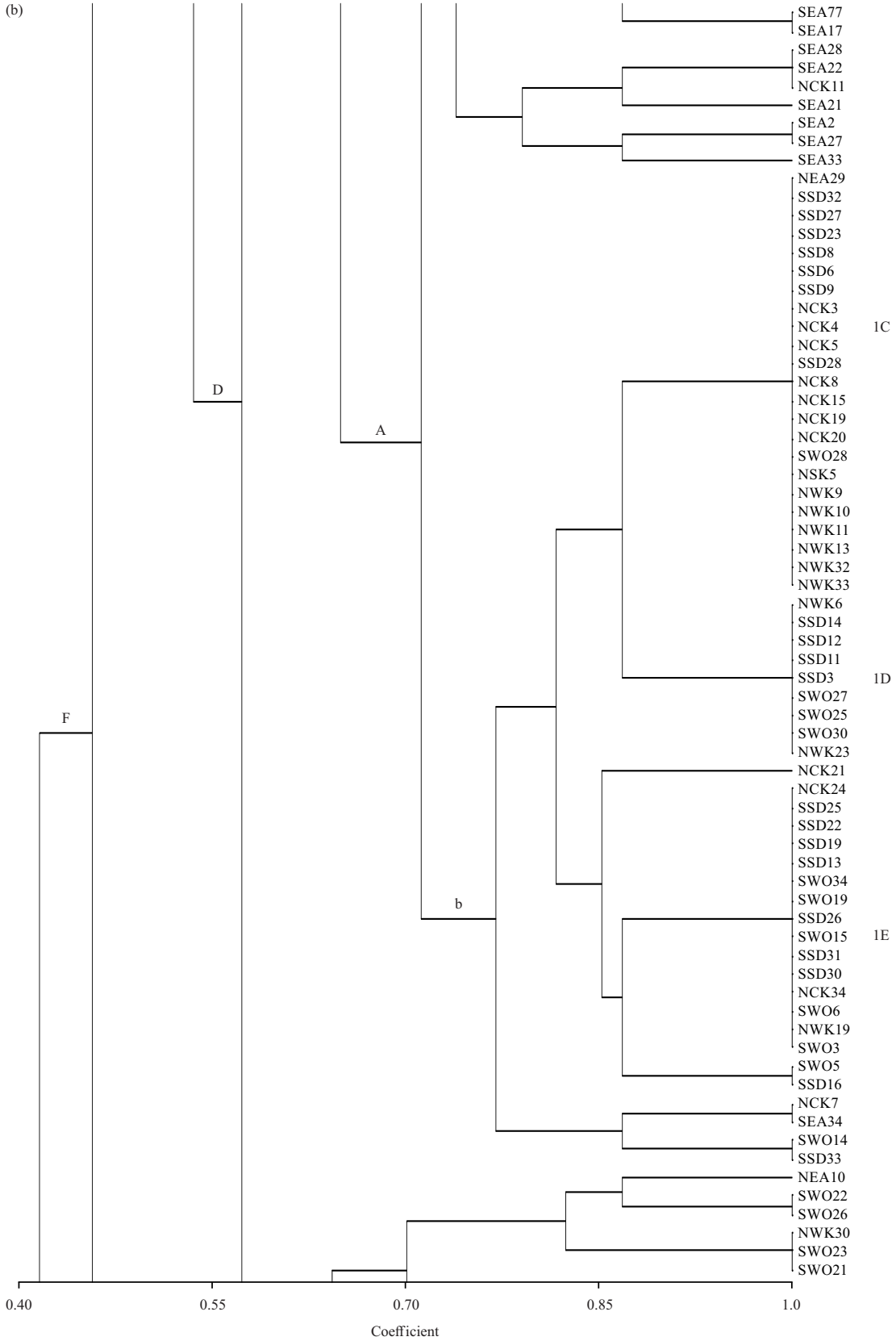

Fig. 2(a-c): Continue

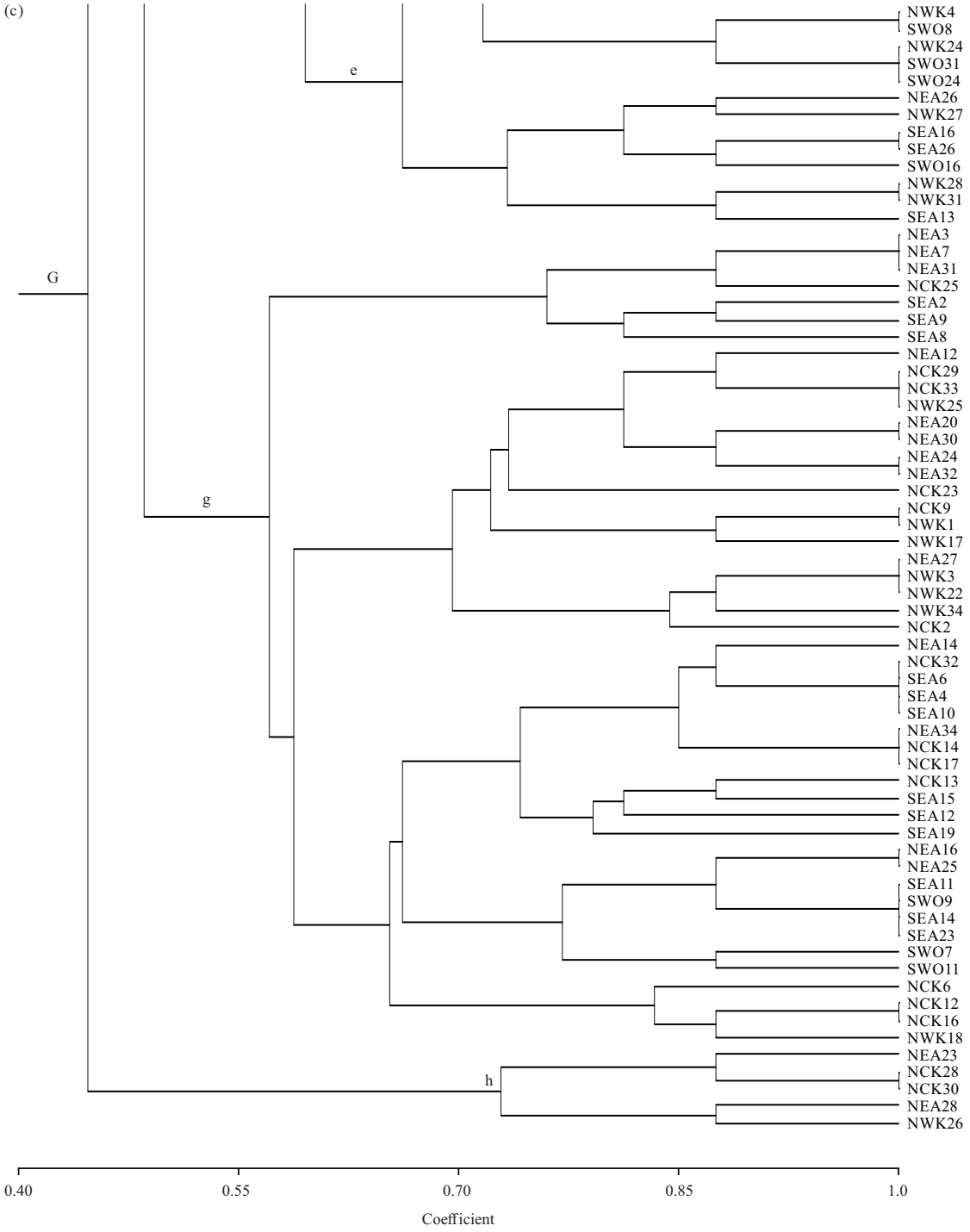

Fig. 2(a-c): Similarity coefficient of among *O. niloticus* (A unit is 0.03)
SSD: South South Delta, SWO: South West Oyo, SEA: South East Anambra, NWK: North West Kebbi, NEA: North East Adamawa and NCK: North Central Kogi

The phylogenetic analysis generated a dendrogram' that showed the similarities and differences between the genes. A total of 118 clusters with varying similarity coefficients ranging from 0.44 to 1 were observed (Fig. 2). Thus, for instance, at a similarity coefficient of 100%, 39 clusters occurred out of which only five were interesting: 1A, 1B, 1C, 1D and 1E clusters (Fig. 2a and b). They were made up of 15, 15, 23, 9 and 15 samples, respectively. Samples from three states in SSD,

SWO and NWK were always present in each cluster at this similarity coefficient. A lot of migration is likely taking place among these fishes over a period. This was in conformity with previous observations of high genetic similarity of *Oreochromis niloticus* species within the Niger River Basin populations over genetically relevant timescales using SNP markers[34].

Seven major clusters of A, B, C, D, E, F, G and sub-clusters a, b, c, d, e, f, g and h were observed. The sub-clusters comprise 10, 6, 5, 3, 6, 10, 28 and 3 clusters at about 73, 77, 73, 79, 66, 62, 56 and 73% similarity coefficients indicating varying levels of similarity. The major clusters showed similarity coefficients of about 73, 67, 64, 59, 56, 48 and 44%, respectively (Fig. 2c). Hence, at cluster G, the level of genetic similarity (44%) within the population is low. Shared bands among *O. niloticus* in the six geopolitical zones were as high as 73%, hence, there was a high similarity among some strains although primers may differ[35]. These high similarity levels may be a result of a major event like flooding which is common in Nigeria or escapes from restricted areas (farms). *Oreochromis niloticus* used in previous work to assess mitochondria diversity of some cichlids in Nigeria also showed varying levels of similarity[36]. However, varying levels of similarity could be an indication that the species is hybridizing with other tilapia species. The *Oreochromis niloticus* like other cichlid species can easily hybridize even with other congeneric and produce fertile offspring that at times have the *O. niloticus* phenotype[37-39]. The DNA fingerprints of mixed DNA samples have been reported to be useful in assessing the relationship between closely related populations due to the high-level genetic differentiation detected[4].

These data supported the view that the very high similarity between tilapia leads to a high probability of hybridization between them, based on the genetic distance between strains we may deduce that there are varying levels of genetic variability among *O. niloticus* in Nigeria water bodies under study. Genetic variability among *O. niloticus* observed in this study was consistent with previous studies of this species using both mtDNA and nuclear markers[17,22,33,35,40-42].

CONCLUSION

Oreochromis niloticus collected from water bodies in six states representing the geopolitical zones of Nigeria show various levels of genetic similarity. The similarity of intensity may be due to the increased amount of hybridization that occurs in the wild among these species. This study findings recommend the use of a more robust genomic approach like the exon gene-capture method in addressing the issues of hybridization.

SIGNIFICANCE STATEMENT

Oreochromis niloticus is well utilized for aquaculture in Nigeria and globally with possibilities of imported strains even hybridizing in the wild when they escape from the farm or through flooding. Our findings using microsatellite markers confirms varying levels of genetic similarities and migration among wild *Oreochromis niloticus* species from the six geopolitical zones of Nigeria. Thereby proving that though pure individuals exist and are spread by events like flooding, introgression might be occurring. Aquaculture and fisheries are faced with challenges, including a lack of pure stock or good seeds to improve fish production. I suggest a robust molecular approach based on a gene capture to ascertain the introgressive hybridization or purity of this species before use in aquaculture or research.

ACKNOWLEDGMENTS

Mojekwu, T.O. appreciates the genomic mapping of Commercially Important Fishes in Nigerian Waters Project (CARGS project) with identification number RFA No. 3 (2) of the Agricultural Research Council of Nigeria (ARCN) for funding this project. Thanks to NIOMR, the Executive Director, the Head of the Department of Biotechnology, Dr. K. Oresegun and Dr. I. Megbowon, the project team leader for the support, time and advice given to T.O. Mojekwu during this work.

REFERENCES

1. FAO, 2020. The State of World Fisheries and Aquaculture 2020. FAO, Rome, Italy, ISBN-13: 978-92-5-132692-3, Pages: 244.
2. Mert, R. and E. Cicek, 2010. Range expansion of introduced tilapia species (*Oreochromis niloticus,* L. 1758, Cichlidae) in Turkey. J. Anim. Vet. Adv., 9: 1753-1756.
3. Yakubu, A.F., N.A. Nwogu, J.O. Apochi, E.D. Olaji and T.E. Adams, 2014. Economic profitability of Nile tilapia (*Oreochromis niloticus* Linnaeus 1757) in semi flow through culture system. J. Aquat. Sci., Vol. 2: 1-4.
4. Allentoft, M.E., R. Heller, R.N. Holdaway and M. Bunce, 2015. Ancient DNA microsatellite analyses of the extinct New Zealand giant moa (*Dinornis robustus*) identify relatives within a single fossil site. Heredity, 115: 481-487.

5. Tripathy, S.K., 2018. Broad spectrum utilities of microsatellite in fish and fisheries. J. Biotechnol. Res., 4: 29-45.
6. Chew, P.C., A. Christianus, J.M. Zudaidy, M.Y. Ina-Salwany, C.M. Chong and S.G. Tan, 2021. Microsatellite characterization of Malaysian mahseer (*Tor* spp.) for improvement of broodstock management and utilization. Animal, Vol. 11. 10.3390/ani11092633.
7. Tibihika, P.D., M. Curto, E. Dornstauder-Schrammel, S. Winter, E. Alemayehu, H. Waidbacher and H. Meimberg, 2019. Application of microsatellite genotyping by sequencing (SSR-GBS) to measure genetic diversity of the East African *Oreochromis niloticus*. Conserv. Genet., 20: 357-372.
8. Vandeputte, M. and P. Haffray, 2014. Parentage assignment with genomic markers: A major advance for understanding and exploiting genetic variation of quantitative traits in farmed aquatic animals. Front. Genet. Vol. 5. 10.3389/fgene.2014.00432.
9. Xiao, T., C. Lu, Y. Xu, C. Li and X. Zheng *et al*., 2015. Screening of SSR markers associated with scale cover pattern and mapped to a genetic linkage map of common carp (*Cyprinus carpio* L.). J. Appl. Genet., 56: 261-269.
10. Briñez, R.B., O.X. Caraballo and V.M. Salazar, 2011. Genetic diversity in six populations of red tilapia, using microsatellites as genetic markers. Rev. MVZ Córdoba, 16: 2491-2498.
11. Ford, A.G.P., T.R. Bullen, L. Pang, M.J. Genner and R. Bills *et al*., 2019. Molecular phylogeny of *Oreochromis* (Cichlidae: Oreochromini) reveals mito-nuclear discordance and multiple colonisation of adverse aquatic environments. Mol. Phylogenet. Evol., 136: 215-226.
12. Vieira, M.L.C., L. Santini, A.L. Diniz and C. de Freitas Munhoz, 2016. Microsatellite markers: What they mean and why they are so useful. Genet. Mol. Biol., 39: 312-328.
13. Sundaray, J.K., K.D. Rasal, V. Chakrapani, P. Swain and D. Kumar *et al*., 2016. Simple sequence repeats (SSRs) markers in fish genomic research and their acceleration via next-generation sequencing and computational approaches. Aquacult. Int., 24: 1089-1102.
14. Chi, J.R., C.W. Huang, J.L. Wu and S.Y. Hu, 2014. Prolactin I microsatellite as genetic markers for characterization of five *Oreochromis tilapia* species and two *Oreochromis niloticus* strains. J. Aquacult. Res. Dev., Vol. 5. 10.4172/2155-9546.1000251.
15. Tibihika, P.D., M. Curto, E. Alemayehu, H. Waidbacher, C. Masembe, P. Akoll and H. Meimberg, 2020. Molecular genetic diversity and differentiation of Nile tilapia (*Oreochromis niloticus*, L. 1758) in East African natural and stocked populations. BMC Evol. Biol., Vol. 20. 10.1186/s12862-020-1583-0.
16. Karuppannan, K.V., I. Noraida and S. Oyyan, 2013. An assessment on red tilapia stocks in Malaysia using microsatellite markers. Int. J. Fish. Aquacult., 5: 78-82.
17. Anane-Taabeah, G., E.A. Frimpong and E. Hallerman, 2019. Aquaculture-mediated invasion of the genetically improved farmed tilapia (gift) into the lower Volta Basin of Ghana. Diversity, Vol. 11. 10.3390/d11100188.
18. Diyie, R.L., S.K. Agyarkwa, E. Armah, N.A. Amonoo, I. Owusu-Frimpong and M.Y. Osei-Atweneboana, 2021. Genetic variations among different generations and cultured populations of Nile tilapia (*Oreochromis niloticus*) in Ghana: Application of microsatellite markers. Aquaculture, Vol. 544. 10.1016/j.aquaculture.2021.737070.
19. Kudo, Y., M. Nikaido, A. Kondo, H. Suzuki, K. Yoshida, K. Kikuchi and N. Okada, 2015. A microsatellite-based genetic linkage map and putative sex-determining genomic regions in lake Victoria cichlids. Gene, 560: 156-164.
20. Liu, F., F. Sun, J. Li, J.H. Xia, G. Lin, R.J. Tu and G.H. Yue, 2013. A microsatellite-based linkage map of salt tolerant tilapia (*Oreochromis mossambicus* x *Oreochromis* spp.) and mapping of sex-determining loci. BMC Genomics, Vol. 14. 10.1186/1471-2164-14-58.
21. Liu, F., F. Sun, J.H. Xia, J. Li and G.H. Fu *et al*., 2014. A genome scan revealed significant associations of growth traits with a major QTL and GHR2 in tilapia. Sci. Rep., Vol. 4. 10.1038/srep07256.
22. Syaifudin, M., M. Bekaert, J.B. Taggart, K.L. Bartie and S. Wehner *et al*., 2019. Species-specific marker discovery in tilapia. Sci. Rep., Vol. 9. 10.1038/s41598-019-48339-2.
23. Lin, G., E. Chua, L. Orban and G.H. Yue, 2016. Mapping QTL for sex and growth traits in salt-tolerant tilapia (*Oreochromis* spp. X *O. mossambicus*). PLoS ONE, Vol. 11. 10.1371/journal.pone.0166723.
24. Chen, C.C., C.W. Huang, C.Y. Lin, C.H. Ho and H.N. Pham *et al*., 2022. Development of disease-resistance-associated microsatellite DNA markers for selective breeding of tilapia (*Oreochromis* spp.) farmed in Taiwan. Genes, Vol. 13. 10.3390/genes13010099.
25. Zhu, H.P., Z.G. Liu, M.X. Lu, F.Y. Gao, X.L. Ke and Z.H. Huang, 2015. Screening and identification of microsatellite markers associated with cold tolerance in Nile tilapia *Oreochromis niloticus*. Genet. Mol. Res., 14: 10308-10314.
26. Telles, M.P.C., L.V. Resende, R.P.V. Brondani, R.G Collevatti, M.C. Costa and N.J. Silva Júnior, 2010. Isolation and characterization of microsatellite markers in the armored catfish *Hypostomus gymnorhynchus* (Loricariidae). Genet. Mol. Res., 9: 1770-1774.
27. O'Reilly, P. and J.M. Wright, 1995. The evolving technology of DNA fingerprinting and its application to fisheries and aquaculture. J. Fish Biol., 47: 29-55.
28. Lopera-Barrero, N.M., J.A. Povh, R.P. Ribeiro, P.C. Gomes, C.B. Jacometo and T. da Silva Lopes, 2008. Comparison of DNA extraction protocols of fish fin and larvae samples: Modified salt (NACL) extraction. Cien. Inv. Agr., 35: 77-86.

29. Kocher, T.D., W.J. Lee, H. Sobolewska, D. Penman and B. McAndrew, 1998. A genetic linkage map of a cichlid fish, the Tilapia (*Oreochromis niloticus*). Genetics, 148: 1225-1232.
30. Chen, Z., C. Hu, L. Wang, G. Chen and J. Wang *et al*., 2015. Microsatellite-based genetic structure and differentiation of goldfish (*Carassius auratus*) with sarcoma. Open J. Anim. Sci., 5: 36-43.
31. Doyle, J.M., G. Siegmund, J.D. Ruhl, S.H. Eo and M.C. Hale *et al*., 2013. Microsatellite analyses across three diverse vertebrate transcriptomes (*Acipenser fulvescens*, *Ambystoma tigrinum*, and *Dipodomys spectabilis*). Genome, 56: 407-414.
32. Zhang, C., B. Vornam, K. Volmer, K. Prinz and F. Kleemann *et al*., 2015. Genetic diversity in aspen and its relation to arthropod abundance. Front. Plant Sci., Vol. 5. 10.3389/fpls.2014.00806.
33. Domingos, J.A., C. Smith-Keune and D.R. Jerry, 2014. Fate of genetic diversity within and between generations and implications for DNA parentage analysis in selective breeding of mass spawners: A case study of commercially farmed barramundi, *Lates calcarifer*. Aquaculture, 424-425: 174-182.
34. Lind, C.E., S.K. Agyakwah, F.Y. Attipoe, C. Nugent, R.P.M.A. Crooijmans and A. Toguyeni, 2019. Genetic diversity of Nile tilapia (*Oreochromis niloticus*) throughout West Africa. Sci. Rep., Vol. 9. 10.1038/s41598-019-53295-y.
35. Bardakci, F. and D.O. Skibinski, 1994. Application of the RAPD technique in tilapia fish: Species and subspecies identification. Heredity, 73: 117-123.
36. Agbebi, O.T., C.J. Echefu, I.O. Adeosun, A.H. Ajibade and E.A. Adegbite *et al*., 2016. Mitochondrial diversity and time divergence of commonly cultured cichlids in Nigeria. Br. Biotechnol. J. Vol. 13. 10.9734/BBJ/2016/25470.
37. Meier, J.I., D.A. Marques, S. Mwaiko, C.E. Wagner, L. Excoffier and O. Seehausen, 2017. Ancient hybridization fuels rapid cichlid fish adaptive radiations. Nat. Commun., Vol. 8. 10.1038/ncomms14363.
38. Shechonge, A., B.P. Ngatunga, R. Tamatamah, S.J. Bradbeer and J. Harrington *et al*., 2018. Losing cichlid fish biodiversity: Genetic and morphological homogenization of tilapia following colonization by introduced species. Conserv. Genet., 19: 1199-1209.
39. Stelkens, R.B., K.A. Young and O. Seehausen, 2010. The accumulation of reproductive incompatibilities in African cichlid fish. Evolution, 64: 617-633.
40. Agnèse, J.F., B. Adépo-Gourène, E.K. Abban and Y. Fermon, 1997. Genetic differentiation among natural populations of the Nile tilapia *Oreochromis niloticus* (Teleostei, Cichlidae). Heredity, 79: 88-96.
41. Bezault, E., P. Balaresque, A. Toguyeni, Y. Fermon, H. Araki, J.F. Baroiller and X. Rognon, 2011. Spatial and temporal variation in population genetic structure of wild Nile tilapia (*Oreochromis niloticus*) across Africa. BMC Genet., Vol. 12. 10.1186/1471-2156-12-102.
42. Iyiola, O.A., R.D. Shaibu, S.O. Oladipo, K.O. Kareem and L.M. Nneji *et al*., 2022. Population genetic structure of three cichlids in Ilorin, North-Central Nigeria. Iraqi J. Vet. Med., 46: 1-9.