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Full Length Research Paper

Levels and patterns of genetic diversity in wild *Chrysichthys nigrodigitatus* in the Lagos Lagoon complex

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Mitochondrial DNA control region sequences were used to investigate the genetic diversity of populations of *Chrysichthys nigrodigitatus and Chrysichthys walkeri* in the Lagos Lagoon complex. A total of 34 haplotypes were detected. The genetic diversity among *C. nigrodigitatus* as determined by haplotype and nucleotide diversities were 0.879 ± 0.033 and 0.0131 ± 0.003, respectively and the values were 0.93 ± 0.04 and 0.010 ± 0.0020 for a population of *C. walkeri*. The largest genetic distance was 7.01% between *C. walkeri* from Lagos Lagoon (WAK) and control region sequences of *Chrysichthys nigrodigitatus* samples obtained from different parts of the lagoon complex in 2008 (PRE). Within population differences accounted for 80.41% of total genetic variance in *C. nigrodigitatus*. There was no evidence of decreased genetic diversity in the populations. The mismatch distribution and neutrality test suggest that the effective size of C*. *nigrodigitatus* population has been large and stable for a long period.

Key words: *Chrysichthys nigrodigitatus*, *Chrysichthys walkeri*, Lagos Lagoon complex, mtDNA control region.

INTRODUCTION

The Lagos lagoon is one of several lagoons in West Africa, which stretches from Benin Republic to Nigeria (Hill and Webb, 1958). The lagoon comprises a network of nine lagoons of shallow waters (Ibe, 1988), which covers an area of 208 km^2 (Ekundayo and Akpata, 1978).

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Abbreviations: LAG, *Chrysichthys nigrodigitatus* of Lagos Lagoon; **EPE,** *Chrysichthys nigrodigitatus* of Epe Lagoon; **WAK,** *Chrysichthys walkeri* from Lagos Lagoon; **PRE,** mtDNA control sequences of *Chrysichthys nigrodigitatus* samples obtained from different parts of the lagoon complex; **PCR,** polymerase chain reaction (PCR); **CHOBA,** *Chrysichthys nigrodigitatus* obtained from the New Calabar River at Choba; **NJ,** neighbour-joining.

In recent years, decline in the viable commercial artisanal fishery of the lagoon have been linked to environmental degradation and possible changes in water quality (Oribhabor and Ezenwa, 2005). The lagoon serves as a sink for large quantities of domestic and industrial wastes (Anetakhai et al., 2007; Olarinmoye et al., 2008; Ayoola and Kuton, 2009). Pollution of lagoons, marine and freshwater environments leads to fish mortalities and reduced species diversity with consequences on overall genetic diversity.

Several studies of the lagoon have focused mainly on the biology of the fishes (Fagade and Olaniyan, 1973; Ikusemiju, 1976; Ikusemiju and Olaniyan, 1977). The silver catfish, *Chrysichthys nigrodigitatus* is one of the most economically abundant species found in the Lagos lagoon complex. The silver catfish, *C. nigrodigitatus* (Lacépède) occurs in most of the major rivers and coastal zones of Africa including Nigeria, Senegal, Gambia, Ivory

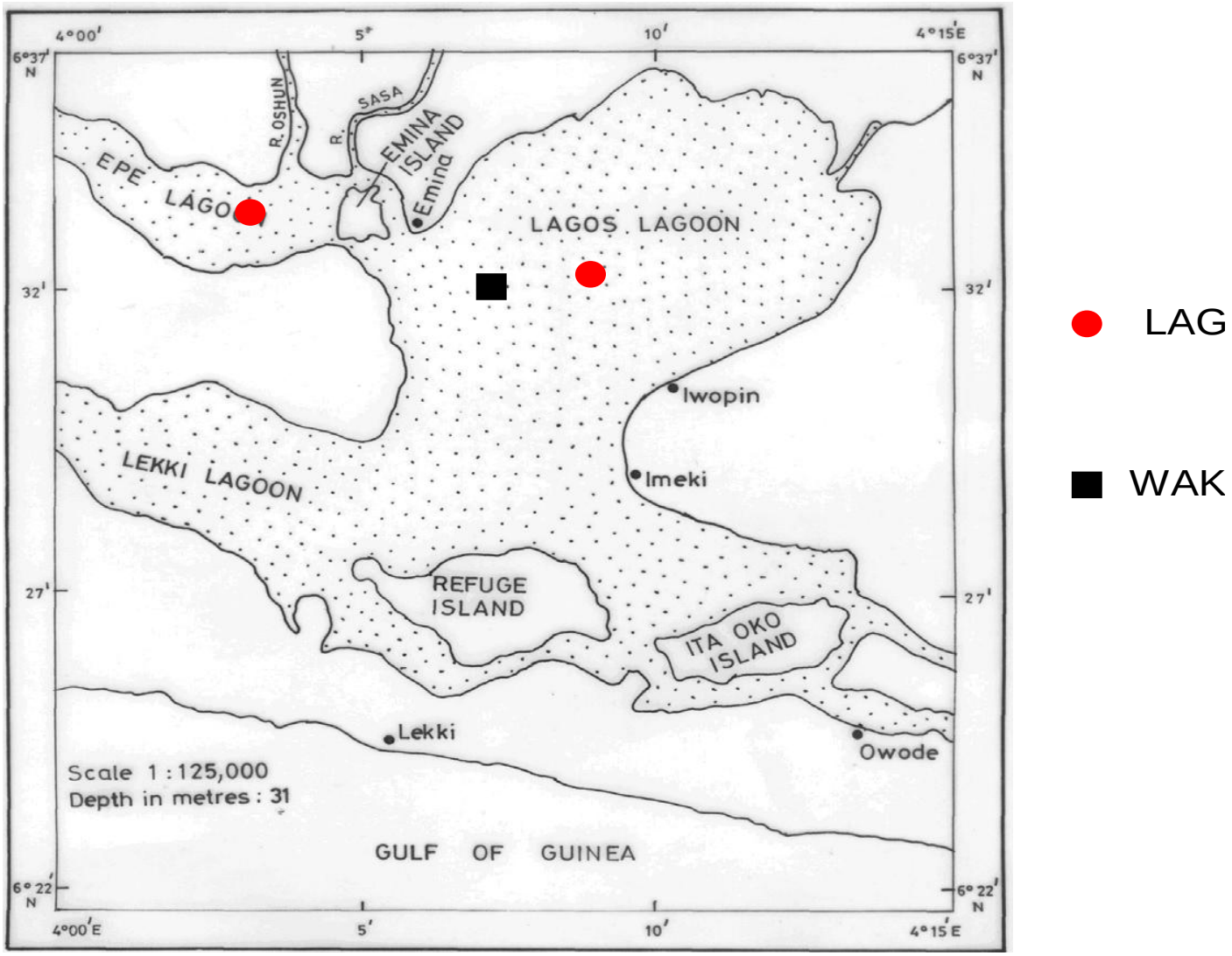

Figure 1. Map of study area showing the sampling sites.

Coast, Liberia, Zaire, and Gabon (Ezenwa, 1981). *C. nigrodigitatus* is a benthic euryhaline teleost fish which migrates to freshwater to spawn, but spends most of its life in estuaries. It makes significant contribution to the artisanal fisheries of the lagoons and its aquaculture potentials are great (Oribhabor and Ezenwa, 2005; Erondu, 1997; Ezenwa et al., 1986). Owing to its economic importance and suitability for culture, considerable research has been devoted to the study of several aspects of the species in Nigerian waters (Ikusemiju and Olaniyan, 1977; Ezenwa, 1981; Anyanwu, 1991; Ekanem, 2000; Offem et al., 2008). However, information on the genetic diversity of the lagoon populations is lacking.

In this study, we examined the genetic diversity of two populations of *C. nigrodigitatus* in the lagoon and compared it with a population of a sibling species, *Chrysichthys walkeri* from which it is almost indistinguishable, by sequencing the most variable portion of the mitochondrial DNA, the control region. We postulate that populations of the Lagos Lagoon complex may have low genetic diversity because of pollution and overfishing. The objectives of the present study were to determine the genetic difference between *C. nigrodigitatus* and *C. walkeri* and if the genetic diversity of *C. nigrodigitatus* from the Lagos Lagoon complex is low.

MATERIALS AND METHODS

Population samples were taken from *C. nigrodigitatus* (n = 52) and *C. walkeri* (n = 25) at two localities (Table 1 and Figure 1). Muscle tissue was sampled from *C. nigrodigitatus* of Lagos Lagoon (LAG) and Epe Lagoon (EPE), while *C. walkeri* was sampled from Lagos Lagoon (WAK). A population of *C. nigrodigitatus* was obtained from the New Calabar River at Choba (CHOBA, not shown in map) and preserved in 95% alcohol before DNA extraction. PRE comprises

Table 1. Sampling data of *C. nigrodigitatus* including population statistics.

ID	Population	N	H	S	Hd	π
LAG	Lagos Lagoon	20	9	44	0.884 ± 0.04	0.017 ± 0.005
EPE	Epe Lagoon	20	11	15	0.884 ± 0.05	0.011 ± 0.001
WAK	Lagos Lagoon	20	12	22	0.930 ± 0.04	0.010 ± 0.002
CHOBA	Calabar River	5	2	1	0.400 ± 0.24	0.001 ± 0.001
PRE	Previous study	12	9	48	0.950 ± 0.05	0.045 ± 0.005
LAG/EPE		40	17	46	0.884 ± 0.03	0.013 ± 0.003

N, Population size; H, number of haplotypes; S, polymorphic sites; Hd, haplotype diversity; π, nucleotide diversity.

mtDNA control sequences of *C. nigrodigitatus* samples obtained from different parts of the lagoon complex during 2008 (Nwafili and Gao, in press).

DNA extraction

Genomic DNA was isolated from muscle tissue by proteinase K digestion followed by a standard phenol-chloroform method. DNA was subsequently resuspended in 100 µl of distilled water for polymerase chain reaction (PCR) amplification.

Amplification reaction was in 50 µl volumes containing 1.25 units of Taq polymerase (TarkaRa, Dalian, China), 200 $nmolL^{-1}$ forward and reverse primers, 200 $\mu molL^{-1}$ each of dNTPs, 10 $mMolL^{-1}$Tris (pH 8.3), 50 $mMolL^{-1}$ KCl and 1.5 $mMolL^{-1}$ $MgCl_2$. PCR amplification was carried out using the primer pair L15923 (5′- TTA AAG CAT CGG TCT TGT AA-3′) and H16500 (5′-GCC CTG AAA TAG GAA CCA GA-3′) (cited in Watanabe and Nishida, 2003) for obtaining the sequence of the first (5′-) half of the mtDNA control region and parts of the threonin (Thr) and proline (Pro) tRNA gene regions. PCR conditions consisted of 35 cycles of denaturation (94°C, 15s), annealing (52 - 55°C, 15 s), and extension (72°C, 30 s) on an Eppendorf 9700 thermal cycler (Applied Biosystems, Foster City, CA, USA). After purifying the PCR products by *Exo*I and SAP treatment (usb Corp., Cleveland, OH, USA) at 37°C, they were sequenced on an automated DNA sequencer (ABI Prism 3700) with amplification primers using the BigDye Terminator Cycle Sequencing FS Ready Reaction Kit (Applied Biosciences).

Data analysis

The partial sequences of the first (5′-) half of the control region including the Thr and proline Pro tRNA gene were compiled with Seqman implemented in DNASTAR software (DNASTAR Inc) and aligned with Clustal X (Thompson et al., 1997) using the default gap penalties. Population genetic statistics such as number of polymorphic sites (S), number of haplotypes (H), haplotype diversity (Hd) and nucleotide diversity (π) were estimated using DnaSP v5 (Librado and Rozas, 2009). The programme Arlequin ver3.1 (Excoffier et al., 2005) was used to search for shared haplotypes between populations. The genetic distances between haplotypes were estimated by using Tamura-Nei model. A neighbour-joining (NJ) tree using Tamura-Nei algorithm was constructed in Mega 2.0 to define the relationship among haplotypes. The robustness of nodes of the NJ distance tree was calculated by bootstrapping (Felsenstein, 1985) with 1000 replicates. *Clarias gariepinus* and *Synodontis nigrita* were used as outgroup. To gain insight into the demographic history of these populations, we used mismatch distribution analysis implemented in Arlquin 3.1 to describe the pairwise differences among individuals within a population. Two tests of neutrality, Tajima's D statistic (Tajima, 1989) and Fu F*s* (Fu 1997), were used to detect signatures of population demographic changes (bottlenecks or expansions) and deviations from the pattern of polymorphism expected from a neutral model of evolution. The examination of deviation from neutrality by both *D* and *F*s indices was based on 1000 coalescent simulations. Analysis of molecular variance (AMOVA) was performed in Arlequin to estimate pairwise population genetic distances and partition genetic variance. CHOBA and WAK were excluded from the AMOVA.

RESULTS

A consensus 522-bp portion of the mitochondrial control region including Thr and proline Pro tRNA gene were obtained. Among 40 sequences of two populations of *C. nigrodigitatus* from EPE and LAG, 46 polymorphic sites were found, which accounted for 8.81% of the total sites and 12 sites were parsimony informative. Seventeen haplotypes were detected (EPE = 11 and LAG = 9). The two populations shared 3 haplotypes (HAP-1, HAP-6 and HAP-12). For PRE, 9 haplotypes were detected out of which haplotypes HAP-1, 4, 12, 17 or 32 were shared with either EPE or LAG. In WAK, 12 haplotypes were found from 20 individuals. Surprisingly, WAK shared haplotypes Hap_21 and Hap_24 with PRE. CHOBA (5 individuals) had only 2 haplotypes, which were not shared with any other population.

Table 1 lists population genetic statistics for *C. nigrodigitatus* and *C. walkeri* based on the mtDNA control region sequences. Overall, haplotype and nucleotide diversities among 40 individuals of *C. nigrodigitatus* were 0.879 ± 0.033 and 0.0131 ± 0.003, respectively. Haplotype diversity for EPE and LAG was the same (0.884) but differs in nucleotide diversity values (Table 1). PRE had the highest value for nucleotide diversity followed by LAG. Overall, genetic diversity indices were high in all populations including WAK (Hd, 0.930 ± 0.04 and π, 0.010 ± 0.002) with values comparable to those of *Chrysichthys macropomum* (Santos et al., 2007), *Brycon opalinus* (Hilsdorf et al., 2001), *Leporinus elongates* (Martins et al., 2003) and *Prochilodus lineatus* (Sivasundar et al., 2001).

The Tamura-Nei's distance (not shown) between EPE and LAG was -0.03% and the largest distance was between EPE and CHOBA (7.46%) followed by 7.01%

between EPE and WAK. The result shows that WAK and CHOBA were very close. Pairwise F_{ST} values demonstrated that LAG was not significantly different from either EPE or PRE ($P > 0.05$) but differs significantly from CHOBA and WAK ($P < 0.01$). Results of AMOVA analysis indicate that genetic variation among *C. nigrodigitatus* populations including EPE, LAG and PRE accounted for 19.59% of the total variation, while 80.41% occurred within populations. Therefore, within population differences was the main source of genetic variation.

The phylogenetic relationship of haplotypes constructed using Tamura-Nei's distances is shown in Figure 2. Two matrilineal lineages supported by high bootstrap value corresponding to *C. nigrodigitatus* and *C. walkeri* were formed. The minimum spanning tree (not shown) also shows how the haplotypes clustered into two main clades. Both the phylogenetic tree and minimum spanning tree showed the existence of significant genealogical structure. The mismatch distribution for each population of *C. nigrodigitatus* is shown in Figures 3 and 4. The Epe population mismatch distribution is bimodal, while that of LAG is multimodal under the constant population size model, indicating that the populations have not always remained stationary. The multimodal mismatch distribution for the LAG population was the result of one divergent haplotype, which appears to be WAK mtDNA because it nested with WAK. Tajima's D and Fu's F*S* were, respectively, 1.06883 ($P > 0.05$) and -1.40165 ($P > 0.05$) for EPE and -1.13016 ($P > 0.05$) and 1.29118 ($P > 0.05$) for LAG.

DISCUSSION

Molecular markers could be veritable tools for monitoring aquatic pollution. Overfishing and pollution of the aquatic environment result in fish mortalities, reduced species diversity and overall loss of genetic diversity because of smaller effective population size. Several workers have indicated that the Lagos Lagoon complex is highly polluted from industrial and anthropogenic activities (Ekundayo, 1977; Akpata and Ekundayo, 1978; Akpata, 1987). The population density around the Lagos Lagoon complex is high resulting in much pressure on the Lagoon. However, Mulvey et al. (2003) found no evidence of decreased diversity in the mtDNA control region of *Fundulus heteroclitus* populations in a highly contaminated environment. Microsatellite analysis of five populations of *C. nigrodigitatus* excluding any Nigerian population revealed high levels genetic variability comparable to those of marine species (Kotoulas et al., 1991). Furthermore, the results of mitochondrial DNA d-loop of *C. nigrodigitatus* samples from the Niger Delta revealed similar extent of differentiation of genetic diversity among population (Nwafili and Gao, in press). In the present study, the three populations of *C. nigrodigitatus* and *C. walkeri* population exhibited relatively high levels of haplotype diversity, indicating the presence of a large number of haplotypes within each population. This result is comparable to patterns observed for marine species including *Lophius budegassa* (Charrier et al., 2006), *Merluccius capensis* (von der Heyden et al., 2007) and *Cynoscion acoupa* (Rodrigues et al., 2008); however, it is higher than those observed for two Australian catfish *Neosilurus hyrtlii* and *Porochilus argenteus* (Huey et al., 2006). The high haplotype diversity may be explained by, first, an initially large population size of the species in the Lagoon complex. Secondly, the benthic life of the fish in quiet waters, crevices and holes make their fishing difficult. The introduction of traps made of hollow bamboos has helped to increase catch, but this should be banned because they target also spawning and brooding males. This behaviour, however, may underestimate the availability of the species judging from catches. Beside non-migrant lagoon residents, the lagoons may be receiving recruitment from the several rivers which flow into them thereby increasing the effective population size. This is also evident in the multimodal mismatch distribution in each population suggesting that these populations have not always been stationary. We infer that this is as a result of supplementation or recruitment from the rivers which flow into the Lagoon. The Oshun River, for example, empties directly into the Epe Lagoon (Figure 1). The lowest haplotype and nucleotide diversities recorded in CHOBA may be due to small sample size. However, overall nucleotide diversities were low but higher than for *Brachyplatystoma rousseauxii* (Batista and Alves-Gomes, 2006). Grant and Bowen (1998) proposed that combination of high haplotype and low nucleotide diversities could be due to rapid population expansion after a period of low effective population size. The two populations of *C. nigrodigitatus* shared only three haplotypes. The presence of many private haplotypes between EPE and LAG is an indication that the two populations may be evolving independently.

The pairwise comparison of genetic distance among 34 haplotypes ranged between 0.00 and 10.18%, the greatest distance being between HAP-12 (haplotype shared among PRE, EPE and LAG) and HAP-26 and 27 (WAK), while the distance between EPE and LAG was only 0.02%. Overall, the greatest net mean distance between groups was between CHOBA and shared haplotype from EPE and PRE. The results show that some individuals from PRE may have introgressed mtDNA from WAK or were wrongly identified as *C. nigrodigitatus*. However, among Japanese freshwater bagrids, Watanabe and Nishida (2003) found a divergence of 0.7 to 8.0% and 0.4% to 13.2% sequence divergence among haplotypes of *Lates calcarifer* (Chenoweth et al., 1998) for the same mtDNA region, values comparable to our result. Therefore, combination of both mtDNA and nuclear DNA markers must be

Table 2. AMOVA analyses of three populations of *C. nigrodigitatus* (EPE, LAG and PRE) based on control region sequences.

Source of variation	Degrees of freedoms	Sum of squares	Variance components	Percentage of variation
Among populations	2	58.381	1.388	19.59
Within populations	49	279.231	5.699	80.41
Total	51	337.613	7.087	

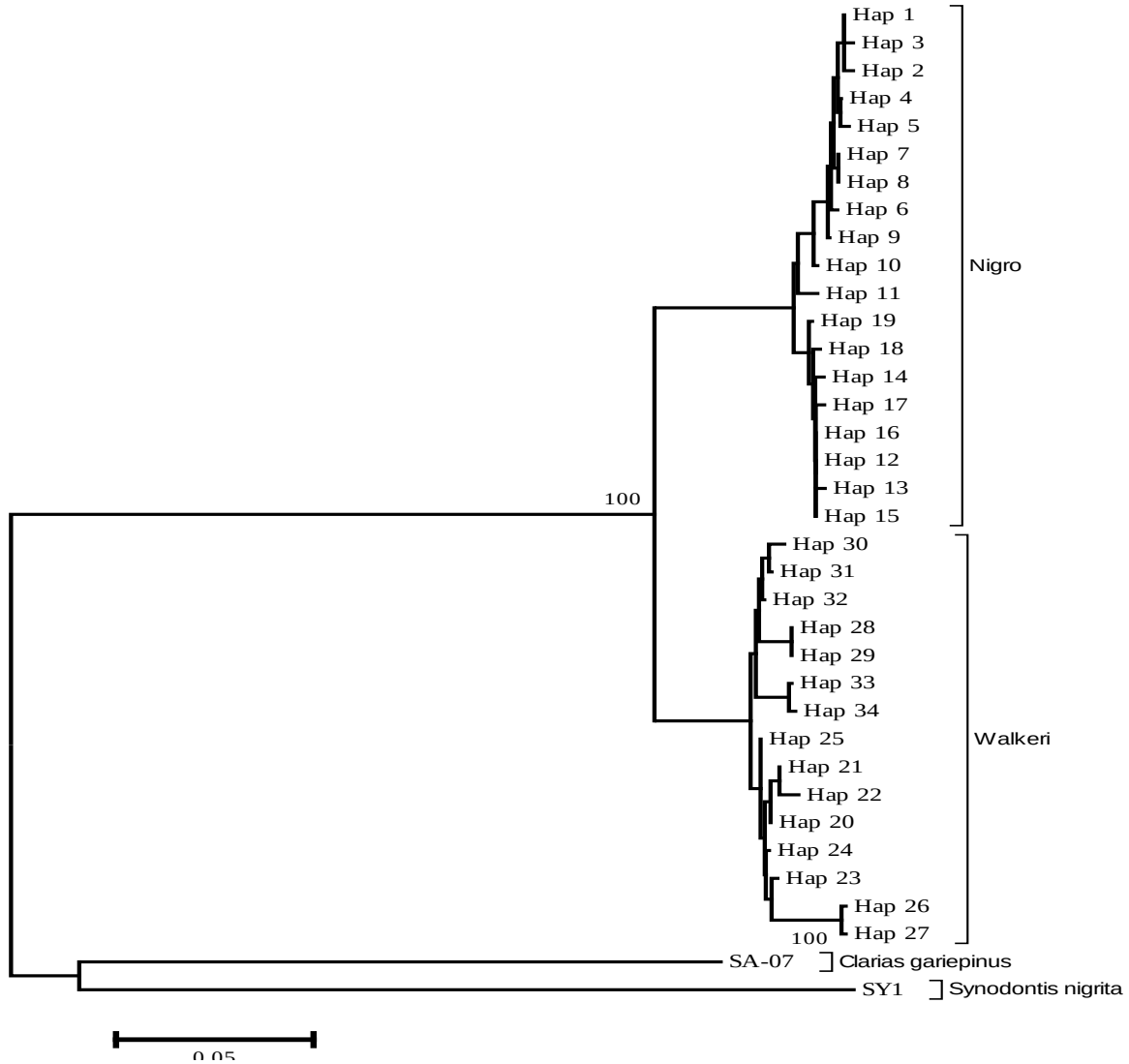

Figure 2. Neighbour-joining tree constructed using Tamura-Nei distances for 34 control region haplotypes of *C. nigrodigitatus* (Nigro) and *C. walkeri* (WAK) from the Lagos Lagoon complex.

employed to resolve this problem.

The AMOVA data show that within population variation significantly accounted for more of the total genetic variation than among populations (Table 2). This is due to the high divergence among PRE populations (Figure 2), some of which nested with WAK. The possibilities are high that some individuals in PRE identified as *C. nigrodigitatus* were *C. walker*i. In the Nigerian coastal lagoons, *C. walkeri* occur with *C. nigrodigitatus* but their morphological differences are not very clear (Sivalingam, 1975). The F_{ST} did not differentiate LAG and EPE. The absence of any obvious physical barrier between Epe and Lagos Lagoon warrants individuals from different parts of the two lagoons to mix and form a panmitic population.

The demographic history showed that populations were

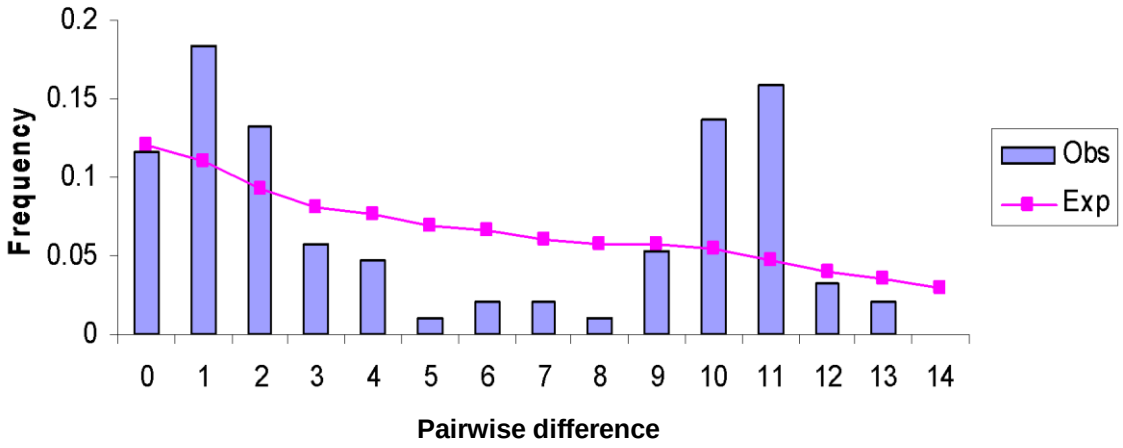

Figure 3. Mismatch distribution analysis for Epe population of *C. nigrodigitatus.*

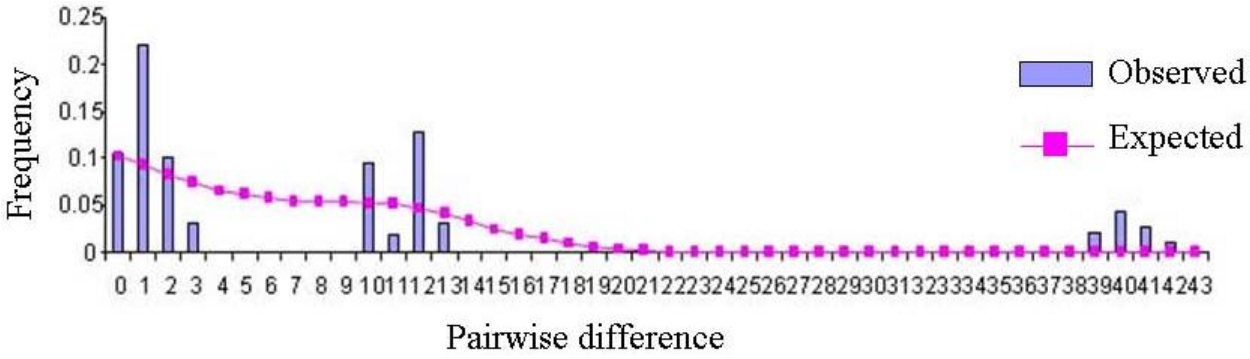

Figure 4. Mismatch distribution analysis for LAG population of *C. nigrodigitatus*

in equilibrium. Non-significant negative Fu's Fs value and positive Tajima's D was obtained for Epe population. Fu's *Fs* is considered to be more sensitive in detecting population expansion and here it suggests possible past population growth in Epe population, but the expansion was weak. The mismatch distribution and neutrality test, however, reject the hypothesis of population expansion in LAG (Figures 3 and 4).

In conclusion, the populations appear genetically healthy and diverse. The Lagos Lagoon complex may present among *C. nigrodigitatus* populations unexpected genetic structure pattern similar to freshwater species and genetic diversity similar to marine species. However, more of the lagoons must be sampled and combinations of nuclear and mtDNA markers employed to reassess the genetic diversity and fine-scale population structure of the species.

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