REGIONAL AND LOCAL-SCALE POPULATION GENETIC STRUCTURE OF A PRIMITIVE TELEOST, THE AFRICAN BONYTONGUE (HETEROTIS NILOTICUS), IN RIVERS OF WEST AFRICA

A Thesis

by

ELIZABETH CARRERA

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

August 2012

Major Subject: Wildlife and Fisheries Science

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	Luis A. Hurtado
Committee Member,	Adam G. Jones
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ABSTRACT

Regional and Local-Scale Population Structure of a Primitive Teleost, the African Bonytongue, (*Heterotis niloticus*), in Rivers of West Africa. (August 2012)

Elizabeth Carrera, B.L.A., Texas A & M University

Co-Chairs of Advisory Committee: Dr. Kirk O. Winemiller
Dr. Luis A. Hurtado

The African bonytongue (*Heterotis niloticus*), one of two living species of the primitive teleost family Arapaimidae, constitutes an important artisanal and commercial fishery in West Africa. This species has also been proposed for wide aquaculture use in Africa. Despite its importance, information on the levels of genetic differentiation for this fish in Benin is lacking, which can contribute to its conservation and management. In this study, regional and local scale genetic differentiation of the African bonytongue in Benin, West Africa, was examined using six microsatellite markers. In total, 221 *H. niloticus* individuals were sampled from 12 localities in Benin that include three river basins: Ouemé-Sô (ten localities sampled); Mono (one locality); and Niger (one locality). The results showed a high degree of genetic differentiation between African bonytongue samples from the three river basins, which was expected given the barriers for dispersal for aquatic organisms. For the Ouemé-Sô floodplain, they indicate high and homogeneous gene flow, suggesting that seasonal flooding facilitates gene flow across this region. The information obtained from this study will be useful for defining management units for *H. niloticus* in Benin, and caution against the translocation of

individuals from different basins.

DEDICATION

I want to dedicate this paper to my parents, my sisters, my grandmother, my uncles and aunts, and also to my advisors, and close friends.

ACKNOWLEDGEMENTS

I would like to thank my committee co-chairs Dr. Kirk O. Winemiller and Dr. Luis A. Hurtado, and my committee member Dr. Adam Jones. My advisors have been the most patient people with me and I persevered thanks to them. I want to give thanks to Dr. John Gold and his lab, especially Mark Renshaw for teaching me how to develop the microsatellite primers. Mark took a lot of time and energy to help me move this project forward. I also want to thank Dr. Dave Portnoy for helping me with specific questions about various genetic software analyses. I also give thanks to Dr. Leslie Winemiller and Dr. Mariana Mateos for their guidance and support throughout the course of this research. I want to express my gratitude to Dr. Alphonse Adite for collecting the samples used in this project. I would like to thank the estate of George and Caroline Kelso for donating funds to this project and allowing it to come into fruition. I want to give thanks members of the Dr. Hurtado and Dr. Mateos laboratory for their help and support. I want to give thanks to my undergraduate research assistant Karina Mankewicz for her laboratory assistance and to Tucker Cullum for technical computer support.

Thanks also go to my best friends, my close friends and colleagues and the department faculty and staff for making my time at Texas A&M University a great experience. Finally, thanks to my mother and father for their encouragement and to my sisters and brother.

NOMENCLATURE

FCA	Factorial Correspondence Analysis
Nm	Effective migration
F_{st}	Proportion of genetic diversity among populations
K	Number of cluster groups
IBD	Isolation by distance
HWE	Hardy Weinberg Equilibrium

SAMPLING LOCALITIES

SOK	(1) Sô River – Near Floodplain
LCOR	(2) Lake Cele-Ephemeral Lake
SOA	(3) Sô River – On Main Channel
LAOR	(4) Lake Azilli – Permanent Large Lake
NT	(5) Niger River Samples
OA	(6) Whedo samples – Modified Ephemeral Ponds
HK	(7) Lake Hlan – Permanent Large Lake
OT	(8) Oueme River- Near Floodplain
LCEOR	(9) Lake Cele – Permanent Large Lake
LNOR	(10) Lake Nakava – Permanent Large Lake
MH	(11) Mono River samples
OKO	(12) Oueme River- On Main Channel

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1. INTRODUCTION*

Inland fisheries are an important source of protein for human consumption in sub-Saharan Africa. In Benin, West Africa, inland fisheries account for ~75% of the fishing activity (FAO 2003). However, increased fishing pressure, use of fishing methods that can be harmful for the sustainability of fish populations, destruction of spawning areas, and reduction of floodplain area for dam construction and agriculture, are threatening inland fisheries in Benin (Hauber et al. 2011). Knowledge required for adequate management and conservation of these fisheries is lacking in many aspects. One of these is the identification of management or conservation units for highly valuable fish species. The African bonytongue, *Heterotis niloticus* (Cuvier 1829), is a fishery that would greatly benefit from this knowledge, due to its importance as a commercial and subsistence fishery across Benin (Gbaguidi & Pfeiffer 1996), and many other countries in Africa, and its potential for aquaculture (Monentcham et al. 2009).

The African bonytongue (*Heterotis niloticus*) is a member of the ancient teleost order Osteoglossiformes and the only member of the family Arapaimidae distributed in Africa (Ferraris 2003, Nelson 2006). According to phylogenetic research (Kumazawa and Nishida 2000) and analysis of fossil records (Lundberg and Chernoff 1992), the

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This thesis follows the style of *Molecular Ecology*.

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closest living relative of *H. niloticus* is the giant arapaima, or pirarucu (*Arapaima gigas*), of the Amazon and Essequibo rivers in South America. Members of Arapaimidae and its sister family Osteoglossidae (arrowanas and saratogas of South America, Southeast Asia and Australia) are collectively referred to as "bonytongues" owing to the ossified tongue that is used to manipulate food within the orobranchial chamber. Both arapaimid species are external spawners and nest guarders; in contrast, all species of the Osteoglossidae are mouth brooders.

The natural geographic distribution of *Heterotis niloticus* spans the Nilo-Sudan region of Africa and includes the Nile, Chad, Niger, Senegal, Gambia, and Volta basins and coastal rivers of Togo and Benin (Nelson 2006). The species also has been introduced for aquaculture at several locations within Lower Guinea (Cameroon, Gabon) and the Congo (Moreau 1982). The African bonytongue inhabits open waters as well as vegetated littoral habitats of lowland rivers as well as lakes and seasonally flooded areas of floodplains (Adite et al. 2005). The species feeds on a variety of food resources, and has been characterized as an omnivore, insectivore, or planktivore by various authors (Lowe-McConnell 1975, Adite *et al*. 2005). The African bonytongue supports important artisanal and commercial fisheries in many regions of Africa. Within West Africa, the species is heavily exploited in southern Benin and the Niger River inland delta in Mali. Annual harvest of bonytongues in Benin was estimated at 742 tons valued at 1,485,000 USD (Gbaguidi & Pfeiffer 1996). Harvest of bonytongues varies seasonally in accordance with fluctuations in river discharge and flooding. Southern Benin experiences two rainy seasons each year that produce a long flood pulse (mid-March to

mid-July) and a short flood pulse (mid-September to October) on the Ouemé-Sô River floodplain (Adite *et al*. 2006).

In the Ouemé-Sô floodplain, *H. niloticus* spawns during the peak of the rainy period but prior to the onset of the major annual flood pulse (May-June) and defend their nests as floodwaters rise (Adite et al. 2006). For several weeks, both parents guard a nest that is constructed within a bed of submerged aquatic macrophytes. Each nest averages about 3,000-5,000 fertilized eggs (Adite *et. al.* 2006). Juvenile *H. niloticus* grow quickly, ~500 grams in 3 months (Monentcham *et al*. 2009). Juveniles disperse into rivers and the flooded plains during the peak of the major flood pulse. Most individuals mature in their second year (Adekeye 1993, Adite et al. 2006). Adite et al. (2006) reported that most of the bonytongues captured in the fishery of the lower Ouemé-Sô River floodplain during the falling-water period were juveniles. In contrast, sexually mature bonytongues were common in Lake Hlan, a natural lake in the Ouemé-Sô River floodplain. Nests and brood-guarding adults were commonly encountered in Lake Hlan, but not in seasonal floodplain habitats, and these observations led these authors to propose that source-sink metapopulation dynamics could be operating in the bonytongue population. Because of local fishing traditions and local control of access to the fishery, Lake Hlan was not exploited as heavily as floodplain areas freely accessed by both local residents and commercial fishers drawn from the broader region. In this case, effective management of bonytongue stocks within the lower Ouemé-Sô River and its extensive floodplain could depend upon maintenance of adult stocks in lakes that export young fish to the floodplain where fishing pressure is greatest. If Lake Hlan and other large permanent

water bodies in the river floodplain are important source areas for production of bonytongue recruits, then these habitats warrant special protections.

Herein, levels of genetic differentiation for *H. niloticus* among three river basins in Benin were examined: Niger River, Mono River, and Ouemé-Sô River (Figure 1). The African bonytongue supports important fisheries in all three basins. Some degree of genetic differentiation is expected among these basins given the geographic barriers that prevent dispersal by aquatic organisms. In addition, fine-scale levels of genetic differentiation were examined within the Ouemé-Sô Rivers floodplain. Panmixia or low levels of genetic differentiation are expected within this basin because extensive seasonal flooding should facilitate dispersal and prevent isolation of local populations.

Objectives

1. To develop microsatellite markers for the African bonytongue (*H. niloticus)* to conduct population genetic analyses in the study area.

2. To test the following hypotheses regarding the degree of genetic structure among the localities collected for *H. niloticus.*

Regional-scale hypotheses

H_0, No significant population genetic differentiation is observed among African bonytongues from the Niger, Mono, and Ouemé-Sô rivers.

H_1, Significant genetic differentiation is observed among African bonytongues from the Niger, Mono, and Ouemé-Sô rivers.

Local-scale hypotheses

H_0, No significant genetic differentiation is observed among localities within the Ouemé-Sô floodplain in southern Benin (i.e., intra-basin panmixia is observed).

H_1, Significant genetic differentiation is observed among localities within the Ouemé-Sô floodplain in southern Benin (i.e., intra-basin genetic structure is observed).

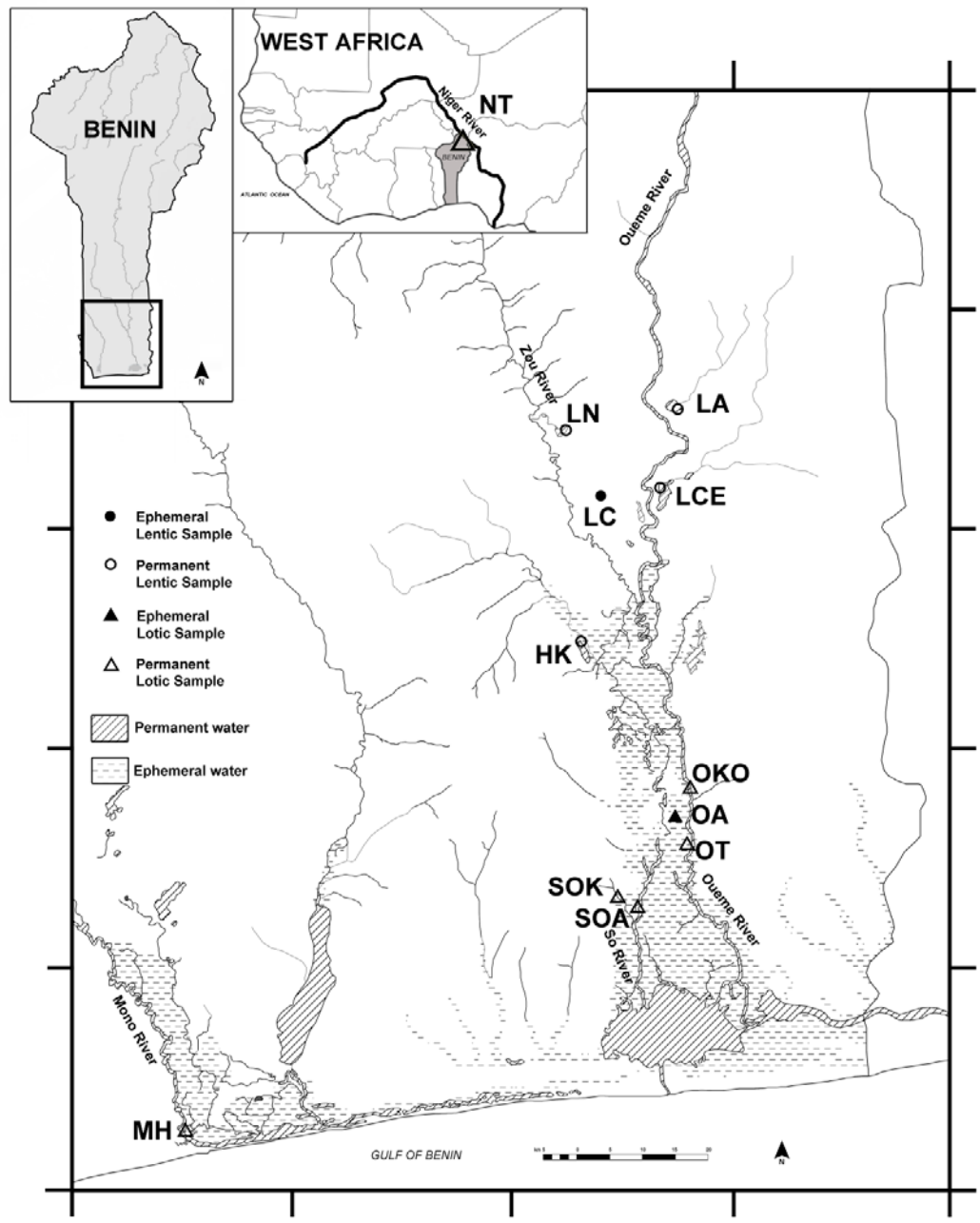

Fig 1. Map of the study area. Map showing the study region in West Africa, MH- Mono River, SOK-Sô River, LCOR- Lake Cele, SOA- Sô River, LAOR- Lake Azilli, NT- Niger River, OA- Whedos, HK- Lake Hlan, OT- Ouemé River, LCEOR- Lake Cele, LNOR- Lake Nakava, OKO- Ouemé River.

2. METHODOLOGY*

In total, 221 *H. niloticus* individuals were sampled from 12 localities in Benin (Fig. 1) that include three river basins: Ouemé-Sô (ten localities sampled); Mono (one locality); and Niger (one locality). Sample size per locality ranged between 6 and 32 individuals (Table 1). Fish were collected during periods of flood pulse recession (Feb.-Mar., Nov.-Dec. in 2008; July 2009 [Niger River]; Feb.-Mar. 2010). The Niger River is the principal river of Western Africa, extending for ~4,180 km. This river crosses the northern portion of Benin, where it corresponds to ~140 km of the border between Benin and Niger. The Mono River is a major river in the eastern part of Togo. Its total length is ~400 km of which ~83 km of its lowest reach correspond to the southern boundary between Benin and Togo. The Ouemé and Sô rivers (the latter being a tributary of the former) flow adjacent to each other on the southern portion of Benin; they share a common floodplain within the coastal plains of Benin. The Ouemé-Sô system drains into Lake Nokue, a large brackish estuary. For each of these rivers, one locality was sampled from the main channel and one from the floodplain near the main channel. Samples also were obtained from five lakes in the Ouemé-Sô floodplain: Lake Hlan, Lake Azilli, Lake Nakava, Lake Cele, and Lake Codo (this last is an ephemeral lake). In addition, a total of 10 juveniles were collected in 2008 and 2009 from six different whedos that were located close to

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each other and the Ouemé River localities (less than 5 km). Whedos are ponds constructed in the floodplain that fill with water during flood pulses when fishes colonize and grow, and then subsequently are harvested during the dry season (Hauber et al. 2012).

Dr. Alphonse Adite and coworkers from the Universite d' Abomey-Calavi collected the field data and fish samples for this study or obtained them from local fishermen. GPS coordinates were recorded at each site, and information was recorded for each specimen, including standard length. Muscle tissue from individual *H. niloticus* was stored separately in small vials with ~85% ethanol and then delivered to Texas A&M University for laboratory analysis.

Table 1. Localities sampled.

Locality	Code	GPS Coordinates	Adult	Young
So River near channel	SOK	06° 35' 43" N ; 002° 23' 15" E;	0	20
Lake Codo	LC	07°07'30"N, 002°20'46"E	12	8
So River on channel	SOA	06° 34' 54" N ; 002° 23' 48" E;	0	10
Lake Azilli	LA	07°15'23"N, 002°27'36"E	6	26
Niger River	NT	11°52'34"N; 003°24'29"E;	0	12
Collective whedos	OA	06°41'44"N; 002°28'04"E;	0	10
Lake Hlan	HK	06°57'07"N ; 002°19'33"E ;	4	32
Ouemé River near channel	OT	06°40'21"N; 002°28'22"E;	13	21
Lake Cele	LCE	07°09'18"N, 002°26'10"E	0	9
Lake Nakava	LN	07°12'25"N, 002°17'40"E	7	10
Mono River	MH	06°18'14"N; 001°50'24"E;	13	2
Ouemé River on channel	OKO	06° 44' 47" N ; 002° 28' 41" E;	5	1
	Total		60	161

Development of microsatellite markers for H. niloticus

DNA was extracted from four specimens from the Ouemé and Niger rivers using the DNeasy Blood and Tissue Kit (Qiagen), following manufacturer instructions and overnight Proteinase-K digestions. DNA from all individuals was combined and the protocol described in Renshaw *et al.* (2009) was used to isolate and characterize microsatellites, with some modifications (Carrera *et al.* 2011). Two 96-well tissue culture plates containing microsatellites were sent to the Interdisciplinary Center for Biotechnology Research at the University of Florida (http://www.biotech.ufl.edu/) for sequencing with universal M13 primers.

A total of 55 candidate loci containing microsatellite motifs were identified. Primers flanking these microsatellite regions were designed using Primer3 (http://frodo.wi.mit.edu/) and purchased from Integrated DNA Technologies (IDT). PCR amplification was tested by screening individuals using 15 µl PCR reactions containing 50–100 ng DNA, 19 µM PCR buffer, 0.5 U Taq DNA polymerase (Genscript), 0.5 µM of each forward and reverse primer (IDT), 200 µM of each dNTP (Genscript), and 2 µM $MgCl_2$ (New England Biolabs). PCR amplifications were carried in a MyCycler (Biorad) and consisted of an initial denaturation at 95°C for 2 min, followed by 38 cycles of denaturation at 95°C for 30 s, annealing at 52.0–59.0°C for 50 s, extension at 72°C for 50 s, and a final extension at 72°C for 10 min (Carrera *et al.* 2011). PCR products were visualized under UV light after electrophoresis on a 1.5% agarose gel stained with Ethidium Bromide. PCR products were obtained for 19 microsatellites. Fluorescently tagged forward primers with either 6-Fam or Hex were purchased from IDT. After

further testing, nine primer combinations consistently amplified PCR products. Information on these primers is summarized in Carrera *et al.* (2011).

Genotyping of localities

All individuals were genotyped for eight microsatellite loci. PCRs were obtained by multiplexing using Qiagen's Multiplex PCR kit, (Multiplex PCR Kit, QIAGEN, Valencia, CA). PCR products were run in an ABI 377 automated sequencer using Genescan 400 HD ROX size standard (Applied Biosystems) for sizing. Allele calling and sizing for each sample was obtained using Applied Biosystem Genescan v.3.1.2 and Genotyper v.2.5 software. To assess quality, PCR and genotyping was repeated for 30% of the samples, which were randomly chosen. All bands were scored manually once by a single person, scored manually again by a different person, and a third time both persons analyzed incongruent calls together to ensure quality control (Selkoe and Toonen 2006). Individual allele genotypes were recorded into a spreadsheet. Only one genotype for one marker from one individual is missing in the entire dataset. The software packages CONVERT (Glaubitz 2004) and PGD SPIDER 2.0.0.3 (Lischer 1996) were used to transform the data into different formats for the subsequent analyses.

Summary statistics

Summary statistics for microsatellite data were obtained, including number of alleles, allelic richness, expected heterozygosity (unbiased gene diversity), and the inbreeding coefficient F_{IS}, measured as Weir and Cockerham's f (Weir and Cockerham 1984), for each locality using FSTAT v. 2.9.3.2 (Goudet 1995). Conformance of genotypes at each microsatellite for each locality to Hardy–Weinberg equilibrium (HWE) expectations

were conducted in GENODIVE (Meirmans and Van Tienderen 2004) using the least squares AMOVA F_{IS} approach, testing 999999 permutations. The software POPGENE was used to test for linkage disequilibrium (i.e., non-random association) between pairs of microsatellite loci, using a Bonferroni correction (Weir 1979). This software was also used to conduct the Ewens-Watterson Test for deviations from neutrality in individual markers (Manly 1985). Presence of potential null alleles was tested using MICRO-CHECKER (Von Oosterhout *et al.* 2004). Mean number of alleles per locus under different sample sizes was estimated for each locality with a rarefaction approach implemented in the software ADZE (Szpiech et al. 2008). Because the amount of observed allelic diversity can be proportional to sample size (Leberg 2002), these results were used to assess whether the estimations of allelic richness in this study are dramatically affected by the sample sizes included for each locality.

Analyses of population genetic differentiation

F_{ST} pairwise genetic distances and chord distances among localities were calculated using the program GENODIVE (Meirmans and Van Tienderen 2004). Isolation by distance for samples from the Ouemé- Sô rivers floodplain was tested using riverine distances and the software program IBDWS (Jensen *et al.* 2005). Additionally, several individual assignment and clustering methods were used to identify clusters or groups among the samples examined.

The first clustering method used is implemented in the program STRUCTURE 2.3.1 (Prichard *et al*. 2000). This is a Bayesian model-based individual clustering method used to examine the number of differentiated populations in a dataset without incorporating a-

priori information of the localities sampled. Analyses were conducted using three iterations for a range of *K* from 2 to 6, with 500,000 steps and a burn-in of 125,000 steps, with all other settings left as default. Convergence on stable likelihoods (i.e., stationary) was determined based on likelihood plots, and on comparison of results from multiple independent runs. Analyses were repeated for four possible model combinations: No Admixture-Correlated frequencies; Admixture-Correlated frequencies; No Admixture-Independent frequencies; and, Admixture-Independent frequencies. To determine the most appropriate value of *K* for each analysis, the method described in Evano et al. (2005) was implemented in STRUCTURE HARVESTER (Earl and VonHolt 2011). Analyses were sequentially repeated eliminating populations that were divergent as suggested by previous analyses.

The second clustering method used was the three-dimensional factorial correspondence analyses (FCA) as implemented in GENETIX (Belkir *et al.* 2000). FCA is a multidimensional statistical method that summarizes large datasets into informative essential subsets that represent the trends of the original data. FCA is adapted for use with diploid genetic data, and it is an exploratory technique suitable for categorical data, which allows investigation of correspondence between rows (e.g., individuals) and columns (e.g., alleles) in a two-way table. It enables visualization of individuals in multidimensional space, with no a priori assumptions about grouping, using each allele as an independent variable. Axes are generated from combinations of alleles that explain portions of the total observed ‘‘inertia’’ of the table. Alleles exhibiting the strongest nonrandom association with groups of individuals will contribute most to the axes.

The third clustering method used was the *K*-means Clustering implemented in GENODIVE. This method clusters sampling localities into an a-priori assigned number of groups (*K*), in a way that within-cluster diversity is minimized and among cluster diversity is maximized. The method uses a pairwise matrix of distances between all observations. Euclidean distances were calculated (as recommended in the GENODIVE manual), which correspond to the Sum of Squared distances from the points to the group's centroid. Clustering was conducted using the hill-climbing method (MacQueen, 1967), which starts by assigning every observation at random to one of the *K* groups and then calculates the error Sum of Squares. A new clustering is then made, by removing one by one each sample, and placing it into the group to which centroid it is closest. The process is repeated and in iteration the new clustering has a smaller Error Sum of Squares until at some point convergence is reached. *K*-means Clustering analyses were conducted for predefined *K* values from 2 to 6. Three methods were used to determine the 'best' *K*: (1) the Akaike Information Criterion, in which the optimal *K* is the one with the lowest value using the Sum of Squares; (2) the Calinski & Harabasz' (1974) pseudo-F-statistic, in which the optimal clustering is the one with the highest value for this statistic; and (3) the Bayesian Information Criterion, in which the optimal clustering is the one with the lowest likelihood value after penalizing the extra number of parameters for the most complex models.

Recent bottleneck analyses

The program BOTTLENECK v. 1.2.02 (Piry et al. 1999) was used to test for signatures of recent bottlenecks in each locality. The principle of the tests implemented in this

program is that after a bottleneck a population exhibits a larger heterozygosity than that expected at mutation-drift equilibrium. This is because allele number is reduced faster than heterozygosity. 'Recent' is defined as within approximately the past 2*Ne*–4*Ne* generations (Piry et al. 1999). Results from the Wilcoxon's test, Sign test, and Standardized Differences test, using the compromise two-phase model (TPM) of microsatellite evolution, are reported. Results from the Mode-Shift test are also reported. This method uses the shape of the allele frequency distribution to differentiate between bottlenecked (shifted-distribution) and stable (L-distribution) populations.

3. RESULTS*

Microsatellite variation and tests

Summary information for the eight polymorphic microsatellite loci for each of the localities examined is shown in Table 2. This includes the number of individuals genotyped, number of alleles detected number of private alleles, allelic richness, observed and expected heterozygosities, probability of deviation from HWE, and F_{IS}. Significant deviations from HWE expectations after Bonferroni correction ($p < 0.0005$) were observed in seven of the 96 calculations. The locus *HNi62* departed significantly from HWE in three localities, the locus *HNi5* in two localities, and the loci *HNi47* and *HNi28* in one locality. No evidence for null alleles or linkage disequilibrium among any pairs of loci was detected. The Ewens-Watterson Test did not find deviations from neutrality at any locus. Due to departures from HWE in more than one locality, loci *HNi62* and *HNi5* were removed for subsequent analyses.

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Table 2. Summary information for eight polymorphic microsatellite loci for each of the twelve *H. niloticus* localities examined in Benin.

	SOK [1]							LCO [2]						
Primer	**N**	**N_A/N_P**	**AR**	**H_O**	**H_E**	**P_{HW}**	**F_{IS}**	**N**	**N_A/N_P**	**AR**	**H_O**	**H_E**	**P_{HW}**	**F_{IS}**
Hni47	20	8/0	4.925	0.800	0.758	0.434	-0.057	20	6/0	4.348	0.800	0.764	0.467	-0.048
Hni94	20	3/0	2.752	0.350	0.447	0.158	0.222	20	4/0	3.159	0.600	0.532	0.314	-0.132
Hni19	20	3/0	2.965	0.550	0.640	0.254	0.143	20	3/0	2.896	0.550	0.553	0.584	0.005
Hni62	20	4/0	2.560	0.500	0.406	0.224	-0.238	20	4/0	3.454	0.500	0.549	0.351	0.091
Hni67	20	4/0	2.115	0.200	0.191	0.848	-0.048	20	4/0	2.637	0.350	0.315	0.532	-0.113
Hni28	20	6/1	5.385	0.700	0.831	0.101	0.161	20	5/0	3.975	0.700	0.708	0.558	0.011
Hni52	20	8/0	5.255	0.800	0.806	0.571	0.008	20	5/0	4.328	0.950	0.769	0.033	-0.243
Hni5	20	6/0	3.890	0.600	0.682	0.271	0.123	20	5/0	3.263	0.650	0.587	0.352	-0.110
	SOA [3]							**LAOR[4]**						
Primer	**N**	**N_A/N_P**	**AR**	**H_O**	**H_E**	**P_{HW}**	**F_{IS}**	**N**	**N_A/N_P**	**AR**	**H_O**	**H_E**	**P_{HW}**	**F_{IS}**
Hni47	10	6/0	5.087	0.900	0.789	0.321	-0.149	32	5/0	3.977	0.594	0.745	0.040	0.205
Hni94	10	2/0	2.000	0.500	0.479	0.718	-0.047	32	5/0	3.427	0.469	0.534	0.204	0.123
Hni19	10	3/0	2.853	0.500	0.595	0.374	0.167	32	5/0	3.303	0.656	0.601	0.297	-0.094
Hni62	10	3/0	2.551	0.400	0.353	0.694	-0.143	32	6/0	4.981	0.344	0.800	0.000**	0.574
Hni67	10	3/0	2.849	0.700	0.532	0.083	0.449	32	5/0	3.799	0.656	0.579	0.141	0.138
Hni28	10	3/0	2.999	1.357	0.700	0.612	0.000	32	7/1	4.722	1.357	0.761	0.097	0.139
Hni52	10	6/0	5.504	0.600	0.837	0.058	0.294	32	7/0	4.987	0.781	0.809	0.412	0.035
Hni5	10	3/0	2.600	0.500	0.574	0.433	0.135	32	10/0	5.501	0.594	0.811	0.002**	0.271

Table 2. (Continued)

	NT [5]						OA [6]						
Primer	**N**	**N_A/N_P**	**AR**	**H_O**	**H_E**	**P_{HW}**	**F_{IS}**	**N**	**N_A/N_P**	**AR**	**H_O**	**H_E**	**P_{HW}**	**F_{IS}**
Hni47	12	3/0	2.522	0.167	0.304	0.090	0.463	10	6/0	4.750	0.700	0.758	0.457	0.080
Hni94	12	1/0	1.000	0.000	0.000	---	n/a	10	4/0	3.200	0.700	0.600	0.359	-0.178
Hni19	12	3/0	2.499	0.083	0.518	0.001**	0.845	10	4/0	3.957	0.600	0.758	0.195	0.217
Hni62	12	4/0	3.214	0.417	0.482	0.366	0.141	10	5/0	4.185	0.500	0.747	0.073	0.343
Hni67	12	5/0	3.951	0.917	0.703	0.066	-0.322	10	3/0	2.200	0.200	0.195	0.947	-0.029
Hni28	12	5/1	3.891	0.833	0.688	0.198	-0.222	10	4/0	3.848	0.700	0.753	0.467	0.074
Hni52	12	1/0	1.000	0.000	0.000	---	n/a	10	4/0	3.551	0.800	0.684	0.326	-0.180
Hni5	12	6/0	4.389	0.333	0.714	0.002**	0.544	10	2/0	1.853	0.000	0.190	0.054	1.000
	HK [7]							**OT [8]**						
Primer	**N**	**N_A/N_P**	**AR**	**H_O**	**H_E**	**P_{HW}**	**F_{IS}**	**N**	**N_A/N_P**	**AR**	**H_O**	**H_E**	**P_{HW}**	**F_{IS}**
Hni47	36	9/0	5.183	0.528	0.791	0.000**	0.336	34	8/0	5.559	0.765	0.814	0.277	0.061
Hni94	36	5/0	3.878	0.694	0.675	0.476	-0.029	34	6/1	3.630	0.588	0.540	0.257	-0.099
Hni19	36	4/0	3.359	0.667	0.563	0.062	-0.187	34	4/0	3.546	0.735	0.626	0.073	-0.177
Hni62	36	9/1	5.317	0.556	0.804	0.000**	0.312	34	10/1	5.864	0.500	0.838	0.000**	0.407
Hni67	36	5/0	3.269	0.389	0.460	0.115	0.157	34	6/1	3.341	0.441	0.512	0.159	0.140
Hni28	36	6/0	3.733	0.750	0.716	0.399	-0.049	34	7/1	4.292	0.647	0.726	0.179	0.110
Hni52	36	8/1	5.285	0.722	0.800	0.153	0.098	34	9/1	5.602	0.882	0.820	0.220	-0.078
Hni5	36	11/0	5.556	0.583	0.789	0.001**	0.263	34	7/0	5.058	0.500	0.795	0.000**	0.375

Table 2. (Continued)

	LCEOR[9]							LNOR [10]						
Primer	**N**	**N_A/N_P**	**AR**	**H_O**	**H_E**	**P_{HW}**	**F_{IS}**	**N**	**N_A/N_P**	**AR**	**H_O**	**H_E**	**P_{HW}**	**F_{IS}**
Hni47	9	4/0	3.873	0.778	0.699	0.460	-0.120	17	7/0	5.286	0.588	0.821	0.018	0.290
Hni94	9	3/0	2.975	0.889	0.620	0.051	-0.471	17	3/0	2.825	0.647	0.528	0.170	-0.235
Hni19	9	4/0	3.799	0.667	0.654	0.655	-0.021	17	3/0	2.725	0.529	0.494	0.505	-0.075
Hni62	9	4/0	3.641	0.444	0.680	0.101	0.360	17	6/0	5.269	0.523	0.827	0.004**	0.367
Hni67	9	3/0	2.667	0.556	0.542	0.653	-0.026	17	5/0	3.987	0.647	0.622	0.546	-0.041
Hni28	9	3/0	2.995	0.556	0.680	0.316	0.192	17	4/0	3.346	0.765	0.706	0.407	-0.086
Hni52	9	6/0	5.602	0.778	0.797	0.584	0.026	17	7/0	5.592	0.529	0.841	0.002**	0.378
Hni5	9	6/0	5.058	0.444	0.758	0.023	0.429	17	10/0	6.214	0.647	0.843	0.028	0.238
	MH [11]							**OKO [12]**						
Primer	**N**	**N_A/N_P**	**AR**	**H_O**	**H_E**	**P_{HW}**	**F_{IS}**	**N**	**N_A/N_P**	**AR**	**H_O**	**H_E**	**P_{HW}**	**F_{IS}**
Hni47	15	6/0	4.232	0.667	0.667	0.623	0.000	6	4/0	4.000	0.333	0.773	0.021	0.592
Hni94	15	4/0	3.528	0.667	0.600	0.386	-0.116	6	3/0	3.000	0.500	0.546	0.516	0.091
Hni19	15	4/0	3.769	0.733	0.699	0.515	-0.051	6	5/0	5.000	0.500	0.803	0.069	0.400
Hni62	15	7/0	5.395	0.800	0.818	0.535	0.023	6	3/0	3.000	0.500	0.667	0.306	0.268
Hni67	15	6/1	4.057	0.467	0.640	0.075	0.277	6	3/0	3.000	0.500	0.621	0.395	0.211
Hni28	15	5/0	2.848	0.133	0.308	0.004**	0.576	6	3/0	3.000	0.333	0.591	0.152	0.459
Hni52	15	4/0	3.735	0.733	0.708	0.545	-0.037	6	3/0	3.000	0.500	0.621	0.410	0.211
Hni5	15	11/2	7.871	0.500	0.923	0.000**	0.468	6	3/0	3.000	0.667	0.530	0.483	-0.290

Indicate significant departures from HWE (Bonferroni correction) <0.005, **N number of samples genotyped, **N_A/N_P** number of alleles and private alleles detected, **AR** allelic richness, **H_O** observed heterozygosity, **H_E** expected heterozygosity, **F_{IS}** inbreeding coefficient

Mean number of alleles and private alleles, heterozygosity, and inbreeding coefficient per locality are shown in Table 3. In general, higher allelic diversity was observed in the localities with larger sample sizes. Ouemé River near the channel (OT; n = 34) and Lake Hlan (n = 36) had the highest allelic diversity with a mean number of alleles equal to 6.67 and 6.17, respectively. Relatively high and medium levels of allelic diversity were observed in Lake Azilli (n = 32), the locality near the Sô River channel (SOK; n = 20), Mono River (n = 15), Lake Nakava (n = 17), and, Lake Codo (n = 20), with a mean number of alleles equal to 5.67, 5.33, 4.88, 4.83, and 4.5, respectively. Lower values of allelic diversity were observed in the whedos-lumped sample (OA; n = 10), Lake Cele (n = 9), the Sô River sample from the channel (SOA; n = 10), the Ouemé River sample from the channel (OKO; n = 6), and the Niger River (n = 12), with a mean number of alleles equal to 4.17, 3.83, 3.83, 3.5, and 3.0, respectively. Despite the apparent relationship between sample size and allelic diversity, rarefaction analyses conducted in ADZE suggest that sample sizes for each locality were sufficient to capture much of the allelic diversity present at each locality (Figure 2).

Table 3 Mean number of alleles and private alleles, heterozygosity, and inbreeding coefficient for each locality.

Population	N_A/N_A	H_O	H_E	F_{IS}
SOK	5.33/1	0.583	0.597	0.076
LCOR	4.50/0	0.608	0.592	-0.087
SOA	3.83/0	0.483	0.623	0.115
LAOR	5.67/1	0.672	0.66	0.094
NT	3.00/1	0.347	0.354	0.101
OA	4.17/0	0.550	0.593	0.013
HK	6.17/1	0.676	0.658	0.065
OT	6.67/4	0.686	0.662	-0.006
LCEOR	3.83/1	0.722	0.629	-0.061
LNOR	4.83/0	0.686	0.649	0.079
MH	4.88/2	0.600	0.583	0.063
OKO	3.5/0	0.722	0.604	0.347

N_A/N_A mean number of alleles detected/Number of private alleles

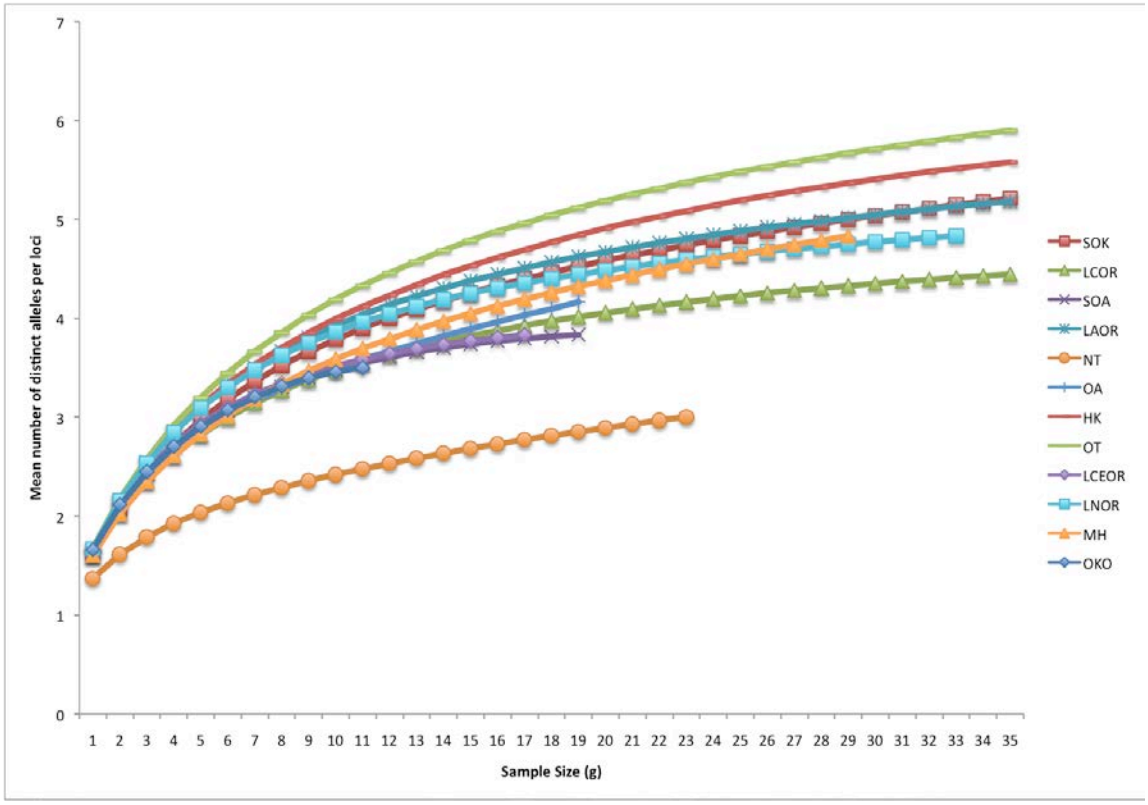

Fig. 2. ADZE analyses. As a function of a standardized sample size, the mean number of distinct alleles per locus are shown for all localities.

Analyses of population genetic differentiation

All pairwise F_{ST} comparisons involving the Niger River locality were significant (Table 4) and these values were the highest observed among the set of comparisons (range from 0.270 to 0.392). All comparisons involving the Mono River locality were also significant with relatively high F_{ST} values (comparisons between Mono River and Ouemé-Sô floodplain localities varied from 0.070 to 0.137). Only three pairwise F_{ST} comparisons between Ouemé-Sô floodplain localities were significant: the locality near the Sô River channel (SOK) and Lake Azilli (LAOR); SOK and Lake Nakava (LN); and the locality near the Ouemé River channel (OT) and Lake Azilli.Significant patterns of isolation by distance (IBD) were observed in only two cases: (1) when all localities were included (p-value = 0.005); and (2) and when all populations except the Niger River population were included (p-value = 0.009).

STRUCTURE analyses are shown in Appendix I. Analyses including all localities with values of K between 2 and 6 (optimal K was between 3 and 5 depending on the model) clearly show that the Niger River locality is differentiated from the other localities, regardless of the model used. Use of the Admixture-Independent model with K = 5 (optimal) also shows differentiation of the Mono River sample. Analyses excluding the sample from the Niger River and analyses excluding Niger River and Mono River localities do not show any clear pattern of differentiation for any locality.

Table 4. Pairwise F_{ST} values between the *H. niloticus* localities examined.

Locality	SOK	LCOR	SOA	LAOR	NT	OA	HK	OT	LCEOR	LNOR	MH
SOK	--										
LCOR	0.011	--									
SOA	0.018	-0.001	--								
LAOR	0.023**	-0.005	0.005	--							
NT	0.345**	0.328**	0.333**	0.270**	--						
OA	0.001	0.004	-0.005	0.022	0.329**	--					
HK	0.017	-0.001	-0.003	0.007	0.271**	-0.005	--				
0T_	0.01	0.017	0.002	0.016**	0.301**	0.003	0.008	--			
LCEOR	0.029	0.018	-0.036	0.019	0.336**	0.005	-0.001	0.008	--		
LNOR	0.033**	0.011	-0.005	0	0.325**	0.038	0.01	0.007	0.01	--	
MH	0.136**	0.137**	0.077**	0.114**	0.392**	0.111**	0.126**	0.085**	0.111**	0.099**	--
OKO	0.044	0.042	0.02	0.019	0.311**	0	0.018	0.008	0.035	0.041	0.070**

**Indicate significant p -values <0.008

The 3D Factorial Correspondence Analysis (FCA) including all localities shows a clear distinction among the three basins sampled: (1) Niger River, (2) Mono River, and (3) the Ouemé-Sô floodplain localities (Figure 3). Analysis excluding the Niger River locality shows a clear distinction between the Mono River sample and the Ouemé-Sô floodplain localities (Figure 4). Finally, in the analysis excluding the Niger River and Mono River localities (Figure 5), some groupings among Ouemé-Sô floodplain localities can be distinguished: (1) the lakes samples (with the exception of Lake Hlan) and the locality in the Sô River channel (SOA); (2) the locality near the Sô River channel (SOK); (3) Lake Hlan and the locality in the Ouemé River channel (OKO); and (4) aggregated whedos samples and the locality near the Ouemé River channel (OT).

Clusters identified by the *K*- means clustering using $K = 3$, which represented the best clustering according to Akaike Information Criterion, correspond to the three river basins: (1) Niger River, (2) Mono River, and (3) the Ouemé-Sô floodplain localities. For $K = 4$, which represented the best clustering according to Calinski & Harabasz' pseudo-F, the clusters identified were: (1) Niger River, (2) Mono River, (3) the locality in the Ouemé River channel (OKO), and (4) all other localities. Finally, for $K = 6$, which represents the best clustering according to the Bayesian Information Criterion, the clusters identified were: (1) Niger River, (2) Mono River, (3) the locality in the Ouemé River channel (OKO), (4) Lake Cele and the locality in the Sô River channel (SOA), (5) Lake Codo, Lake Azilli, Lake Hlan and Lake Nakava, and (6) the locality near the Sô River channel (SOK) and the two localities in the Ouemé River (OA and OT).

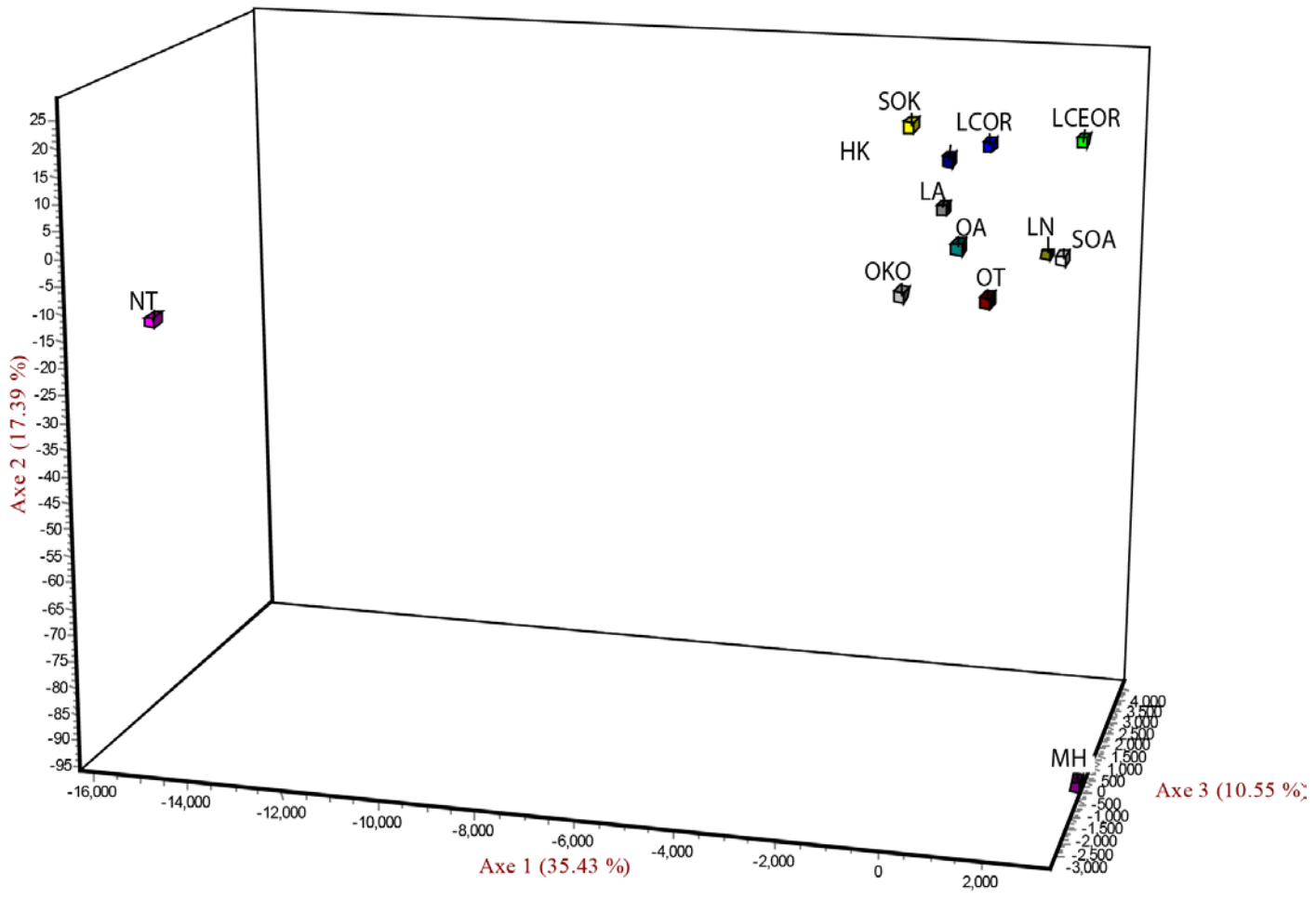

Fig. 3. 3D Factorial Correspondence Analysis (FCA) including all localities.

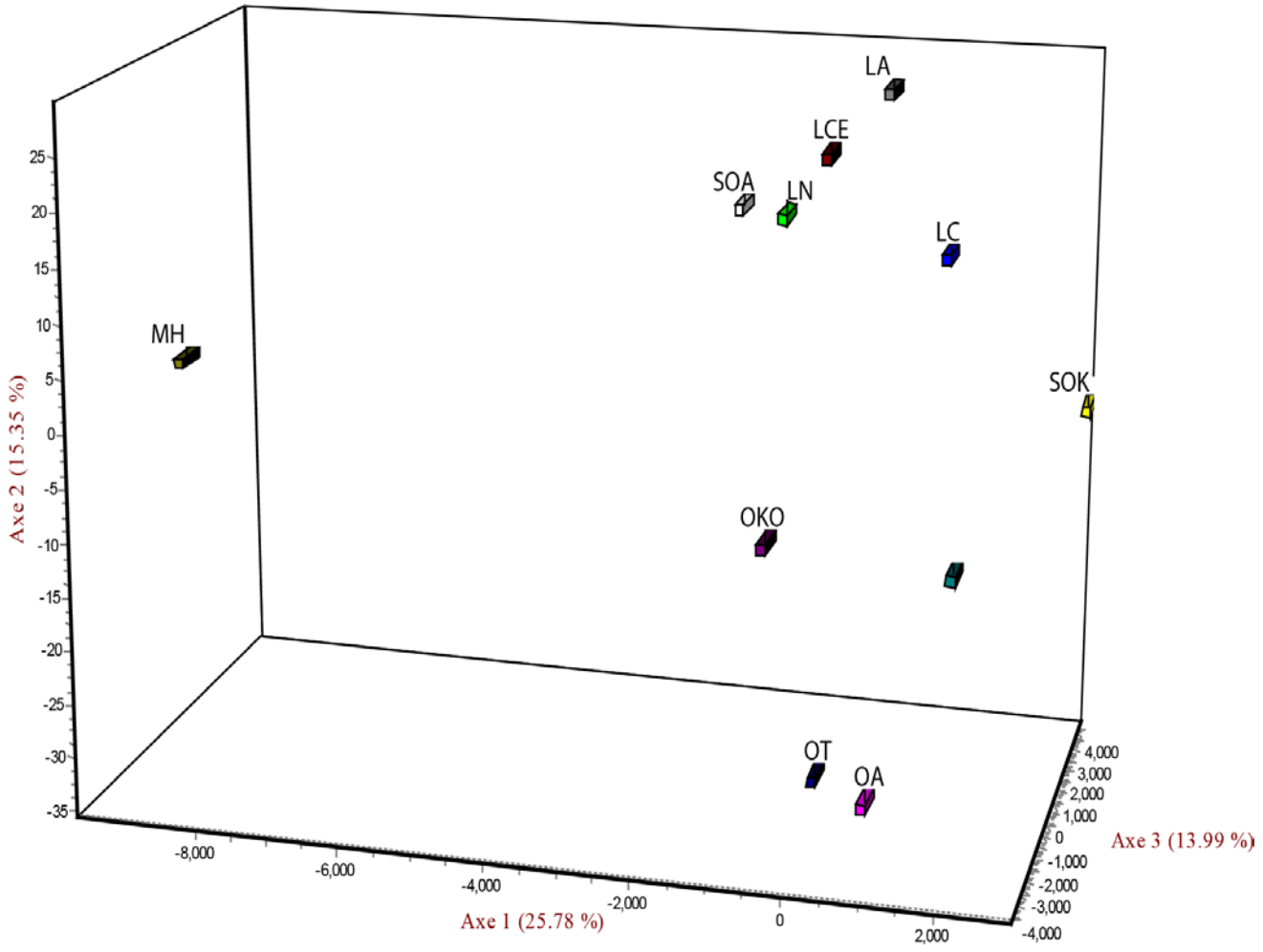

Fig. 4. 3D-FCA excluding the Niger River locality.

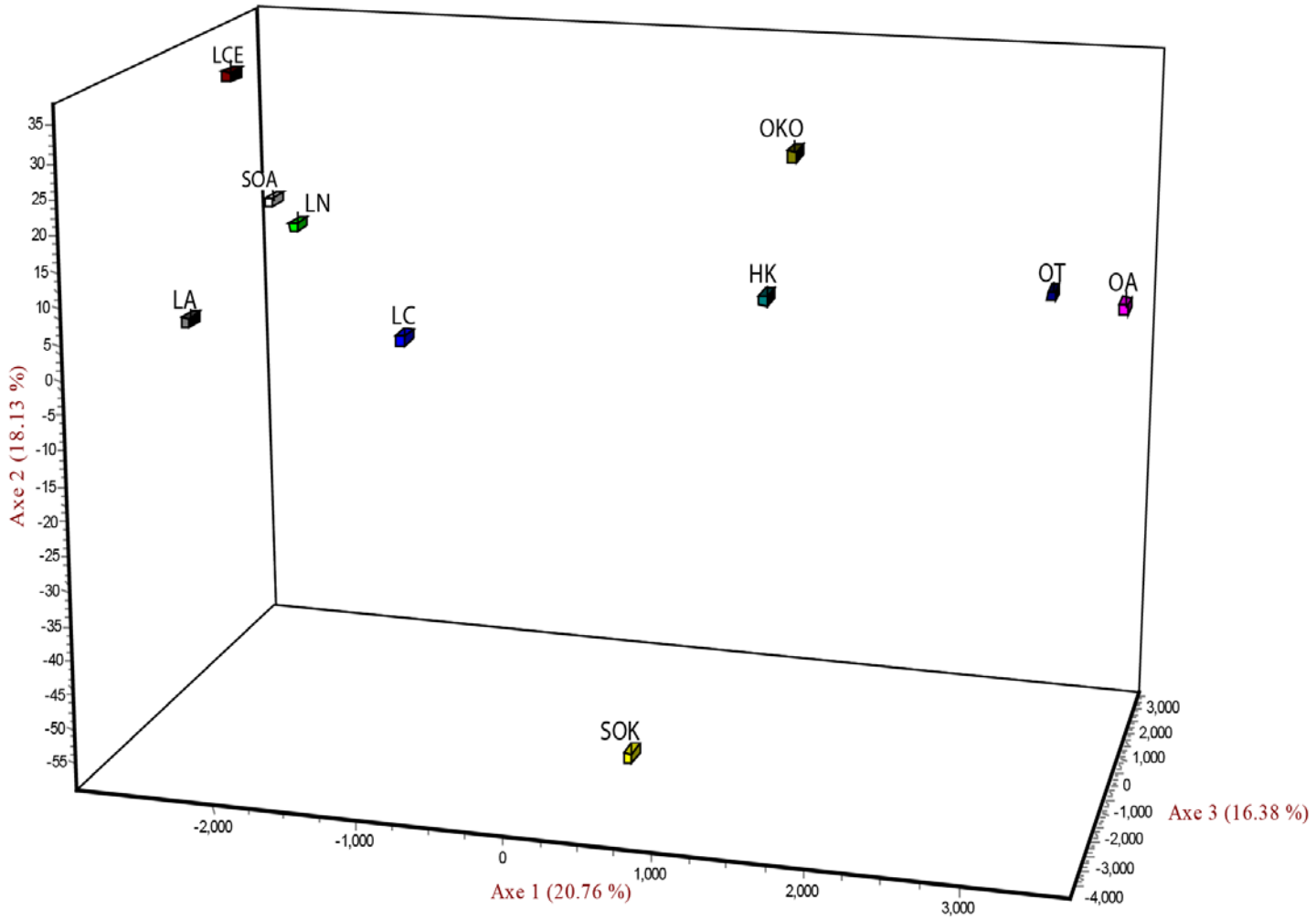

Fig. 5. 3D-FCA excluding the Niger River and Mono River localities.

Recent bottleneck analyses

Signatures of recent bottlenecks were detected by more than one test in Lake Cele (LCE), the locality in the Sô River channel (SOA), and the locality in the Ouemé River channel (OKO). For Lake Nakava (LN) only the Wilcoxon's test was significant(Table 5). No signatures of recent bottlenecks for any test were detected among the remaining localities.

Table 5. Recent bottleneck tests.

Locality	Wilcoxon test	Sign test	Std. difference test	Mode Shift
SOK	0.422	0.529	0.429	Left shift
LCOR	0.281	0.527	0.364	Left shift
SOA	**0.008**	**0.034**	**0.023**	**Shifted**
LAOR	0.281	0.475	0.285	Left shift
NT	0.56	0.544	0.339	Left shift
OA	0.422	0.484	0.442	Left shift
HK	0.218	0.223	0.388	Left shift
OT	0.578	0.544	0.324	Left shift
LCE	**0.008**	**0.043**	0.083	**Shifted**
LN	**0.023**	0.210	0.085	Left shift
MH	0.719	0.475	0.071	Left shift
OKO	**0.016**	0.204	0.142	**Shifted**

Bolded numbers indicate significant at $p < 0.05$

4. DISCUSSION

The results showed a high degree of genetic differentiation between African bonytongue samples from the three river basins examined (i.e., Niger, Mono and Ouemé-Sô). This was expected given the barriers for dispersal for aquatic organisms. In the Ouemé-Sô floodplain, the results suggest a generally high and homogeneous gene flow, although some weak local genetic differentiation may be occurring. Below, the results for each basin and their implications for conservation are discussed.

Niger River

All analyses used to examine population genetic differentiation found that the Niger River locality was highly differentiated from the other localities examined in this study. Distance and barriers to dispersal may explain this high level of genetic differentiation. The Niger River locality is ~600–700 km apart (straight distance) from the other localities examined in this study and no direct water connections between the Niger River locality and the other localities exist. Therefore, a severe restriction of gene flow between this locality and the localities at the other basins is expected.

Based on the high degree of genetic differentiation observed in the Niger River sample, the bonytongue population of this basin should be considered a different management unit (Fraser and Bernatchez 2001). However, an expanded analysis of genetic structure in northern Benin is necessary to determine how many management units are present in this region. As in southern Benin, this species is the basis for important commercial and subsistence fisheries in northern Benin (Hauber et al. 2011;

2012), where, during the peak of the annual flood pulse, the Niger River covers an area of 274 km^2 (Welcomme 1985). Thus, information on the levels of population genetic differentiation will be important for proper management and conservation of bonytongue stocks in this region.

Average allelic diversity and heterozygosity observed (3 and 0.347, respectively) in the Niger River locality were the lowest among all the *H. niloticus* localities sampled in this study. These values are very low in comparison to those reported for other freshwater fishes. A meta-analysis that included 13 species of freshwater fishes, 75 loci, and 7,755 individuals found average allelic diversity and heterozygosity values per species of 9.1 and 0.54, respectively (DeWoody and Avise 2000). The low genetic diversity observed in the Niger River locality suggest that this population may be very vulnerable to overexploitation because inbreeding depression may arise quickly, and that its evolutionary potential may be limited. No genetic evidence for a recent bottleneck was found, although it is possible that our analyses lacked power due to small sample size and number of markers. Interestingly, extremely low genetic variability of *H. niloticus* was also found in mitochondrial genes. Comparison of sequences of a 850 bp fragment of the ND2 mitochondrial gene between individuals of the Niger River locality and the Ouemé-Sô floodplain revealed differences at only two nucleotide positions. It is important to establish whether low genetic microsatellite variability is specific to the Niger locality sampled or it is a characteristic of the northern portion of Benin.

Given that the African bonytongue is widely distributed along the Niger River, it is important to extend genetic surveys to regions above and below the reach that forms part

of the northern border between Benin and Niger. The Benin portion of the Niger River constitutes only a relatively small stretch (~140 km) of this large river that extends 4,180 km, and the river flows through parts of other Western African countries, including Guinea, Mali, Niger, and Nigeria. It is likely that genetic studies of localities sampled throughout the Niger River will reveal population structure. A genetic study of the Arapaima (*Arapaima gigas*), the closest living relative of the African bonytongue (*H. niloticus*), found isolation by distance for this fish in a transect of ~2,700 km in the Amazon River (Hrbek *et al.* 2004).

Mono River

Most of the results also show that the Mono River locality is strongly differentiated from the other localities included in this study. Pairwise F_{ST} values were high and significant in all comparisons including the Mono River. FCA and *K*-means clustering analyses also show a clear distinction of this locality. With the exception of one analysis, STRUCTURE analyses did not show a clear separation of this locality from the Ouemé-Sô floodplain localities. Nevertheless, cases in which STRUCTURE fails to detect population structure when it actually exists have been reported (e.g., Francois and Durand 2010).

Gene flow between the Mono River locality and the Ouemé-Sô floodplain localities could be highly restricted because the locations are separated by more than 50 km without any freshwater connection. Given its genetic differentiation, the Mono River locality should be considered a different management unit. The Mono River is the major river in eastern Togo, extending ~ 400 km, of which ~83 km correspond to the southern

boundary between Benin and Togo. Thus, a more fine-scale analysis of genetic structure along the Mono River would be needed to determine if more than one bonytongue stock are present in this basin.

Average allelic diversity and heterozygosity observed for bonytongues from the Mono River (4.88 and 0.6, respectively) were intermediate relative to values obtained from all localities that were surveyed. Average allelic diversity for bonytongues from the Mono was very low compared to values reported (9.1) for other freshwater fishes (DeWoody and Avise 2000). Nonetheless, average heterozygosity in Mono River bonytongues was higher than values reported (0.54) for other freshwater fishes (DeWoody and Avise 2000). No genetic evidence for a recent bottleneck was found at this locality.

Ouemé-Sô floodplain

The results suggest that, in general, high and homogeneous gene flow occurs for *H. niloticus* in the Ouemé-Sô floodplain. Seasonal flooding undoubtedly facilitates gene flow of this fish across this region. Nonetheless, various population genetics analyses suggest that some degree of weak local differentiation may be occurring. The F_{ST} comparisons and FCA suggest some differentiation for the Sô River channel in the floodplain region (SOK). F_{ST} comparisons also suggest a degree of genetic differentiation for Lake Azilli. Clustering analyses suggest some groupings for different lakes and for some river locations. However, a clear pattern is not evident, instances of genetic differentiation do not appear very pronounced, and no evidence of isolation by distance was found. Furthermore, it is uncertain whether these spatial differences

represent effects of isolation or are the result of stochasticity in the temporal dynamics structuring subunits of *H. niloticus* individuals at the local scale. It is important to note, however, that the power to detect weak differentiation may have been limited by the use of only six microsatellites and the small sample sizes for some of the localities (i.e., OKO, SOA and Lake Cele). Results for the Niger River and Mono River localities, however, indicate that the six microsatellite markers were able to detect significant differences for F_{ST} values as low as 0.077 and a sample size of twelve individuals. Most of the F_{ST} values for the Ouemé-Sô river and floodplain comparisons were < 0.02 (30 out of 36), with a maximum estimated F_{ST} value of 0.038, suggesting that, if present, local differentiation in this region is very weak.

Migration among river, lakes and ephemeral floodplain habitats during the flooding season may prevent local genetic differentiation in the Ouemé-Sô system. Floodplain lakes have been proposed as refugia for African bonytongue population subunits that export migrants to the river and floodplain during the annual flood pulse. Adite et al. (2006) found that Lake Hlan is an important spawning site for bonytongues, where ripe adults comprised over 40% of the *H. niloticus* individuals, whereas only 3.5% of individuals captured from localities in the Sô River and floodplain were adults. They also observed that Lake Hlan contained many large *H. niloticus* breeding adults and small juveniles but few intermediate size classes, whereas these intermediate size classes dominated the river floodplain region to the south. These observations led them to propose that Lake Hlan harbored an important source subpopulation that exports new *H. niloticus* recruits downstream to the Sô River and associated floodplains. Such migration

patterns could help explain the overall genetic homogeneity observed in the Ouemé-Sô system. However, individuals should migrate into as well as out of lakes in order to explain the lack of genetic differentiation observed in lake samples.

Given the low levels of genetic differentiation found, *H. niloticus* within the Ouemé-Sô system appear to constitute a single management unit. Conservation practices in this area should treat different lakes, river channels, and seasonal floodplain pools, as a set of interconnected habitats used by this fish. As suggested by Adite et al. (2006), maintenance of healthy stocks of spawners in the permanent lakes, such as Lake Hlan, probably is essential for sustainable fisheries in the Ouemé-Sô floodplain. In general, population genetic diversity of *H. niloticus* in the Ouemé-Sô floodplain appears to be good. Average allelic diversity was higher in one Ouemé River locality (6.67) and Lake Hlan (6.17), over twice that observed in the Niger River locality (3), but still below the average (9.1) reported for freshwater fishes (DeWoody and Avise 2000). In all but one locality, heterozygosity of bonytongues was higher than the average reported (0.54) for freshwater fishes (DeWoody and Avise 2000). In general, no signatures of recent bottlenecks were found in the Ouemé-Sô system. Evidence of a bottleneck was only detected in two ephemeral floodplain habitats and the Ouemé river channel, but the latter estimate could have been biased by a small sample size (n = 6).

Management and conservation implications

The results have important implications for biodiversity conservation and management of *H. niloticus* fisheries. The African bonytongue supports important fisheries in West Africa and has been introduced in rivers and aquaculture stations across Africa

(Monentcham et al. 2009). For example, the species is reported to have been translocated from northern Cameroon to southern Cameroon, from southern Cameroon to the Central African Republic, Congo and Gabon, from Cameroon to Madagascar and the Ivory Coast, and from the Congo to the Democratic Republic of Congo (Monentcham et al. 2009). In several countries, the establishment of exotic *H. niloticus* populations was reported to have negatively impacted native fish species (Monentcham et al. 2009). The levels of population genetic differentiation observed among the three basins studied in Benin suggest that translocations of this fish from different geographic regions could be harmful for the integrity of 'native' genetically differentiated *H. niloticus* lineages. This may occur if introduced *H. niloticus* have higher fitness than endemic conspecifics, or if genomic introgression by hybridization replaces the genetic make up of local lineages. Extirpation of endemic lineages by competition and hybridization with foreign congeners has been documented in several freshwater fishes. For example, introduction of nonnative smallmouth bass *Micropterus dolomieu* into central Texas streams resulted in introgressive hybridization with the endemic allopatric congener Guadalupe bass *M. treculii* (Littrell et al. 2007). Pure Guadalupe bass are no longer present in these streams, whereas pure smallmouth bass represent 40% of the population, smallmouth bass X Guadalupe bass hybrids represent 51%, and other *Micropterus* hybrids represent 9% (Littrell et al. 2007).

In addition, outbreeding depression could occur if two genetically differentiated lineages of *H. niloticus* hybridize, as has been show in other fish species. For example, outbreeding depression is reported in hybrids of largemouth bass (*Microterus salmoides*)

from two geographically separated populations (i.e., Illinois and Wisconsin) with a small degree of genetic differentiation (F_{ST} = 0.05) and comparable fitness (Goldberg et al 2005). F1 hybrids had a reduction in fitness of ~14%. In addition, experimental trials indicated that two weeks after inoculation with the largemouth bass virus, survival of individuals from parental populations and F1 hybrids was similar (~ 80%), whereas survival of the F2 hybrids was only 35%. Outbreeding depression has also been shown in hybrids between two populations of pink salmon (*Oncorhynchus gorbuscha*) separated by ~1000 km, in which F1 and F2 hybrids had a significant reduction of survival (Gilk et al. 2004). Another study showed that genetic introgression with non-native rainbow trout (*Oncorhynchus mykiss*) affects reproductive success (number of offspring per adult) of native westslope cutthroat trout (*Oncorhynchus clarkii lewisi*) in the wild (Muhfeld et al. 2009). Small amounts of hybridization markedly reduced fitness of male and female trout, with reproductive success sharply declining by approximately 50% with only 20% admixture (Muhfeld et al. 2009). A study of Atlantic salmon (*Salmo salar*) concluded that outbreeding effects are highly variable or unpredictable at small genetic distances and need to be evaluated on a case-by-case basis (Houde et al. 2011). Given the scale at which genetic differentiation was observed for *H. niloticus*, there is potential for outbreeding depression in hybrids of fish originating from different river basins. Potential negative consequences of introgression between separate lineages of *H. niloticus* should be considered for both aquaculture and management of capture fisheries. As observed in other fish species, even low levels of admixture could significantly affect fitness.

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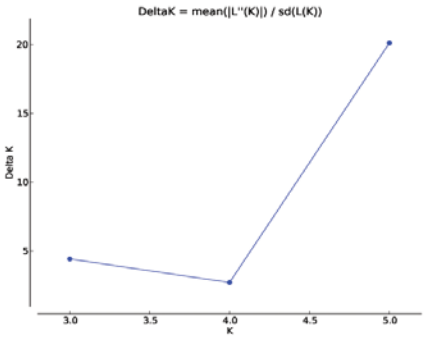
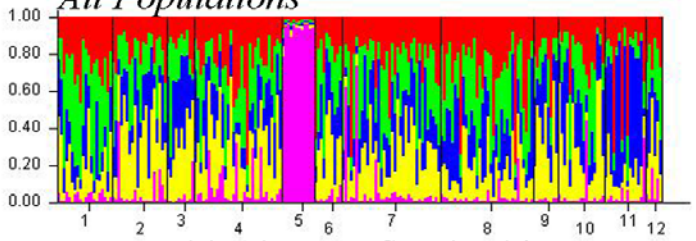
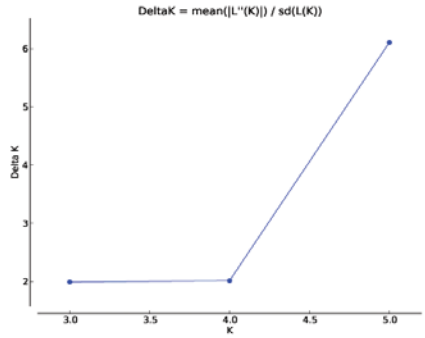
APPENDIX A

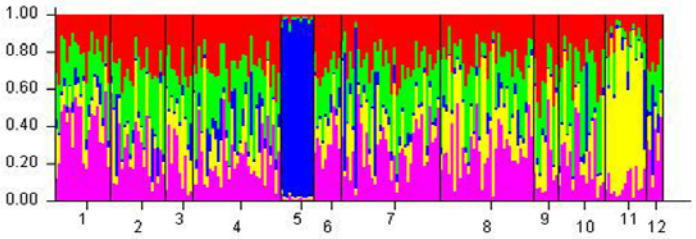


Structure Harvester/ Structure Results- All Populations



Model: Admixture- Correlated frequencies





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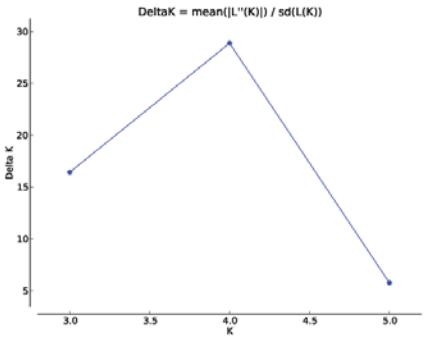

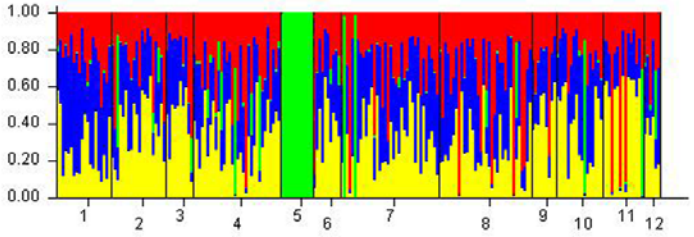

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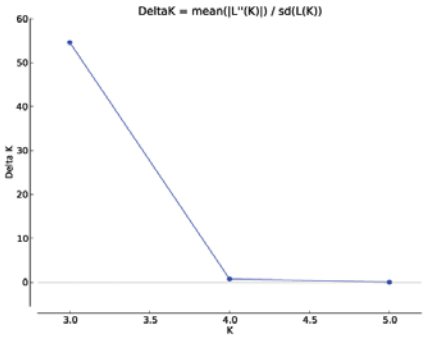

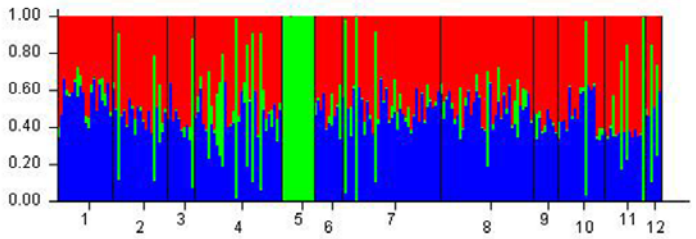

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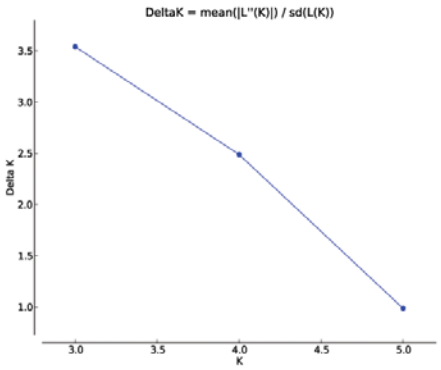
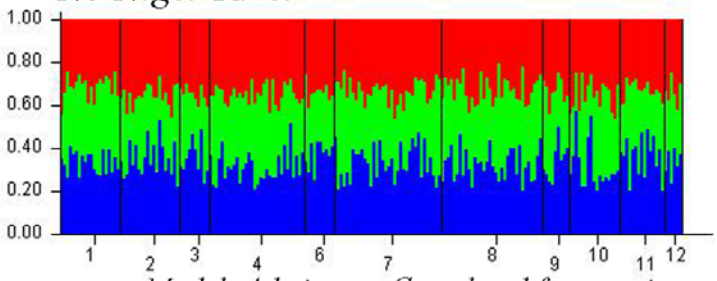


Structure Harvester/ Structure Results
No Niger River



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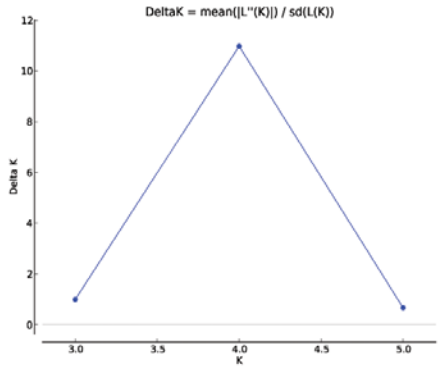
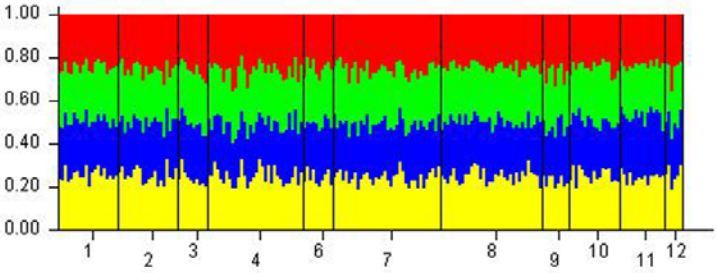
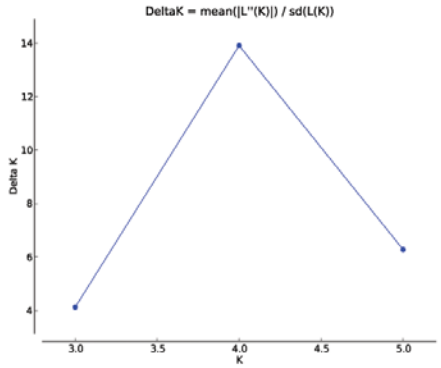
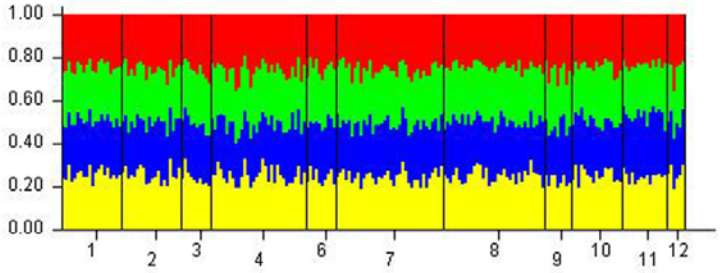


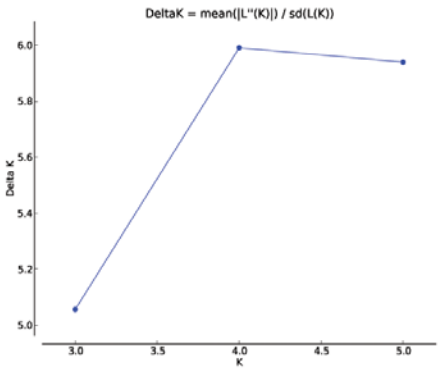


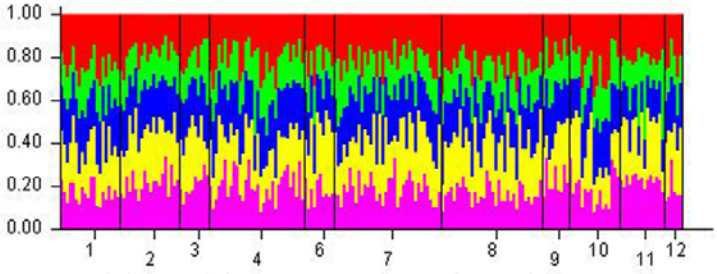
Model: Admixture- Independent of frequencies



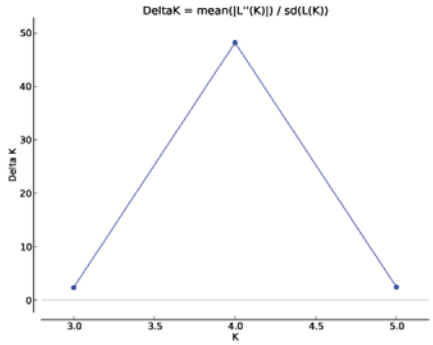


Model: No Admixture- Correlated frequencies

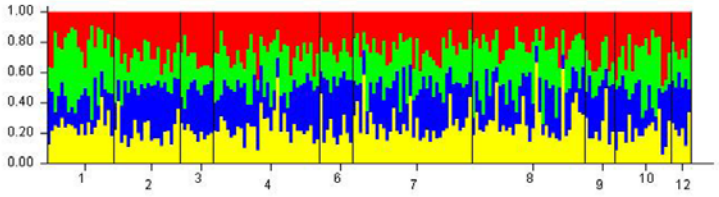




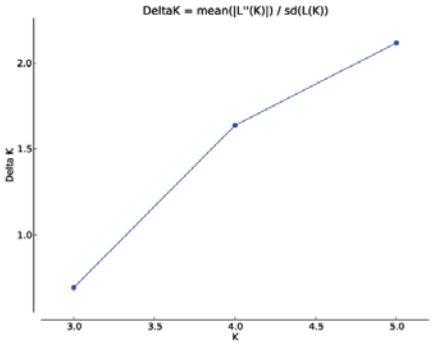
Model: No Admixture- Independent of frequencies

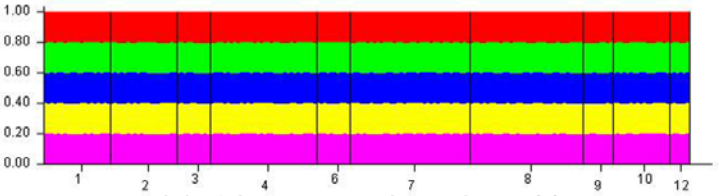


Structure Harvester/ Structure Results
No Niger River, No Mono River

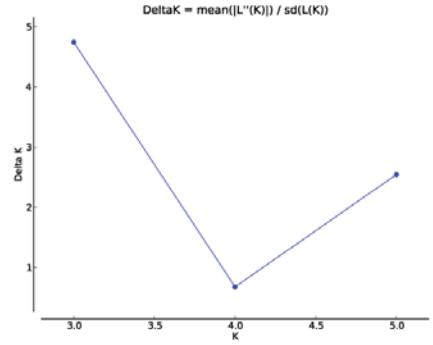


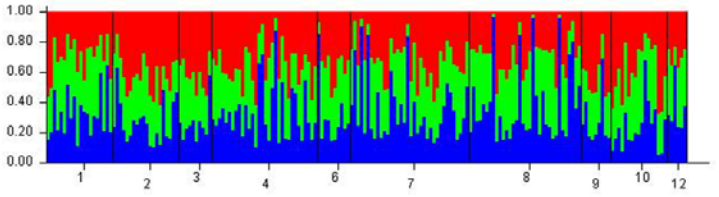
Model: Admixture- Correlated frequencies



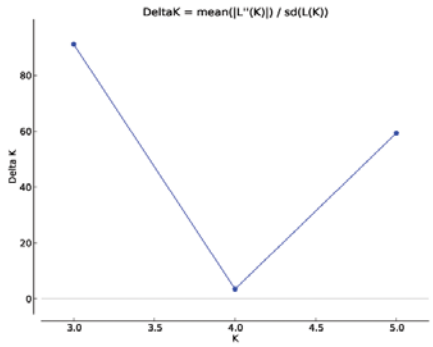


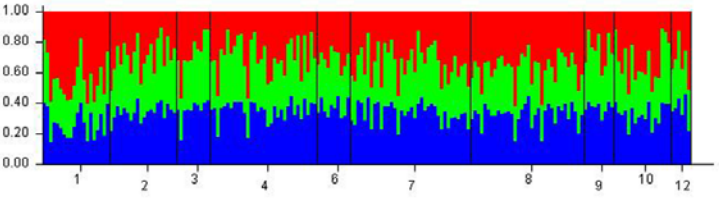
Model: Admixture- Independent of frequencies





Model: No Admixture- Correlated frequencies

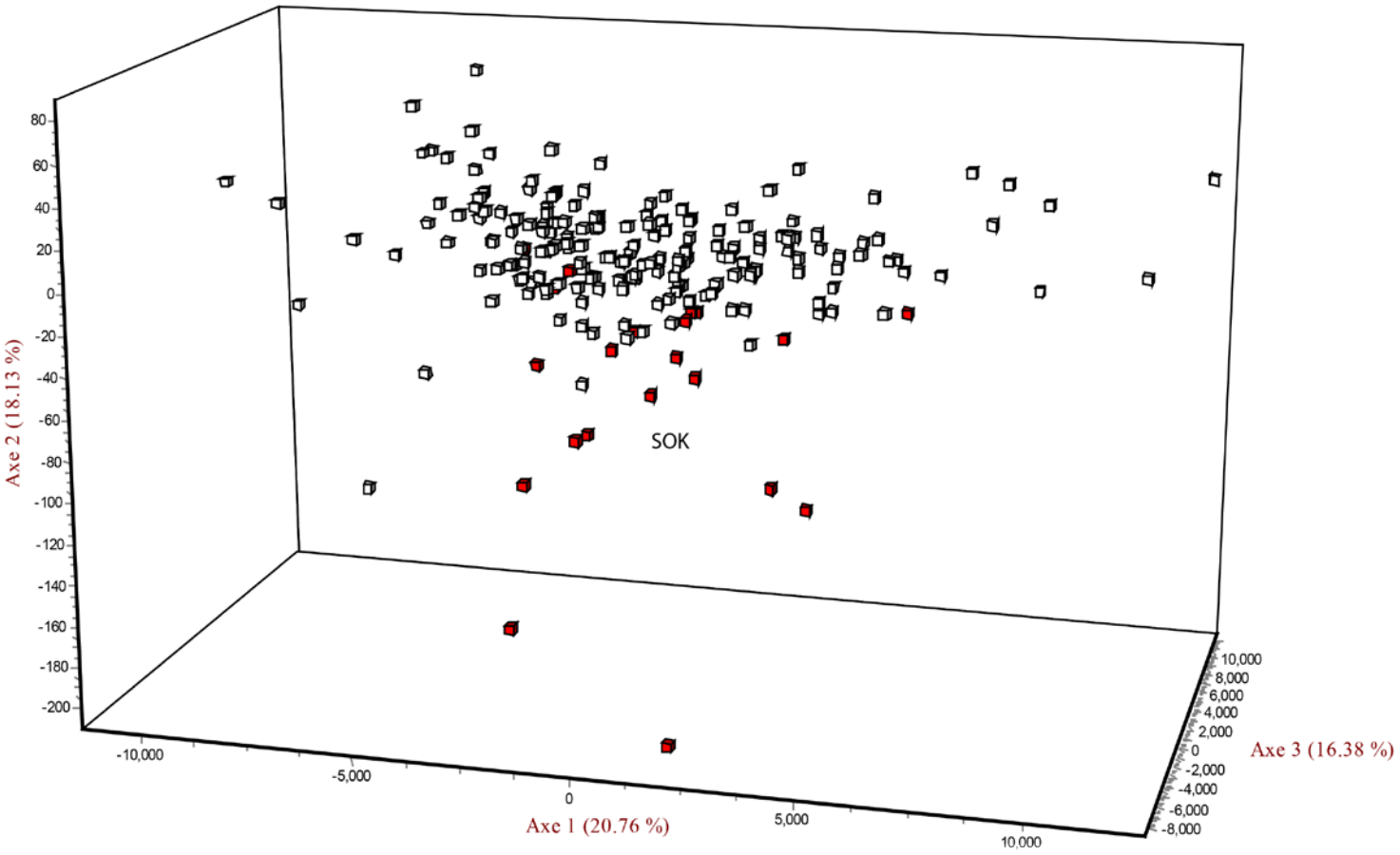


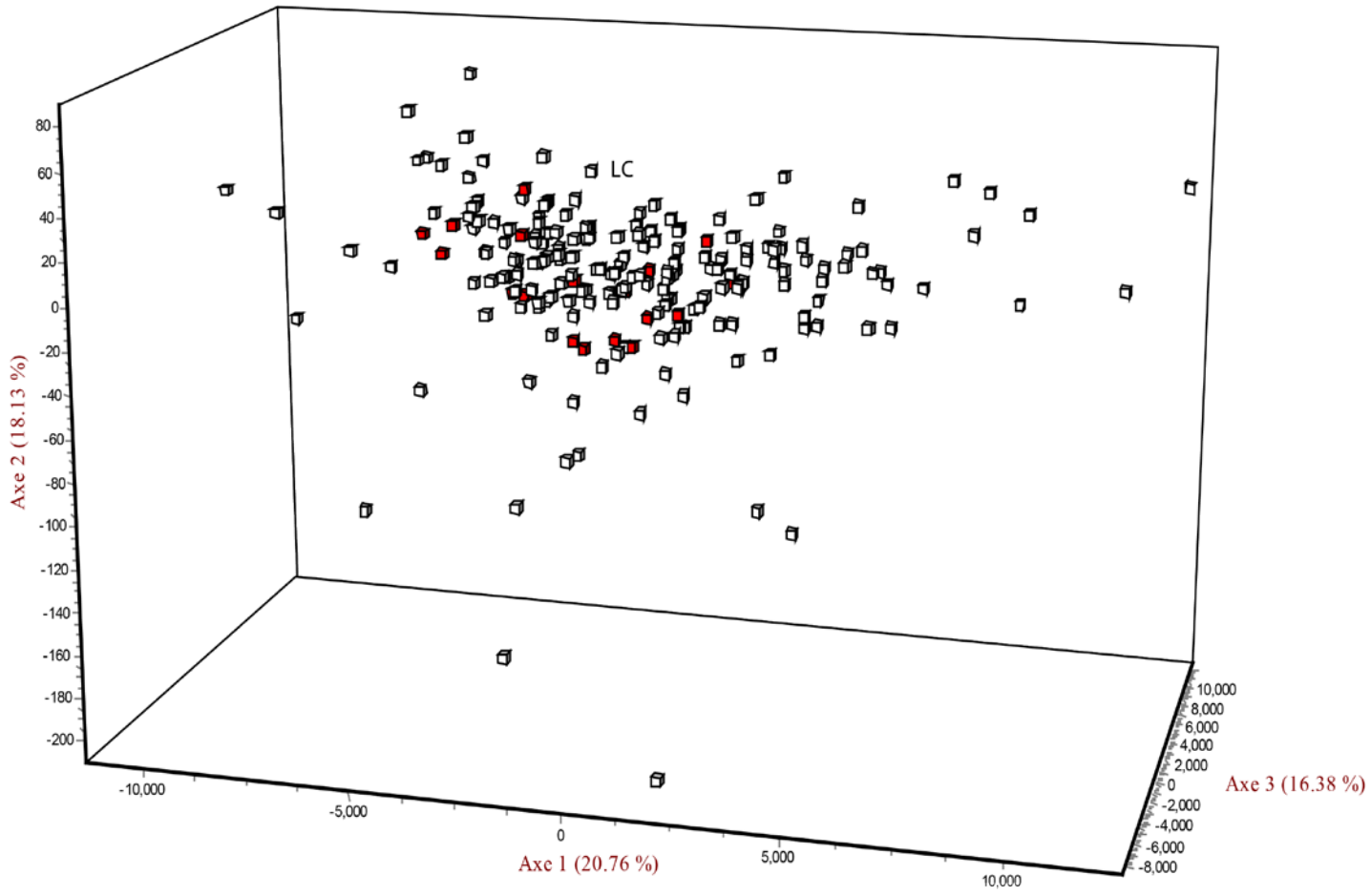


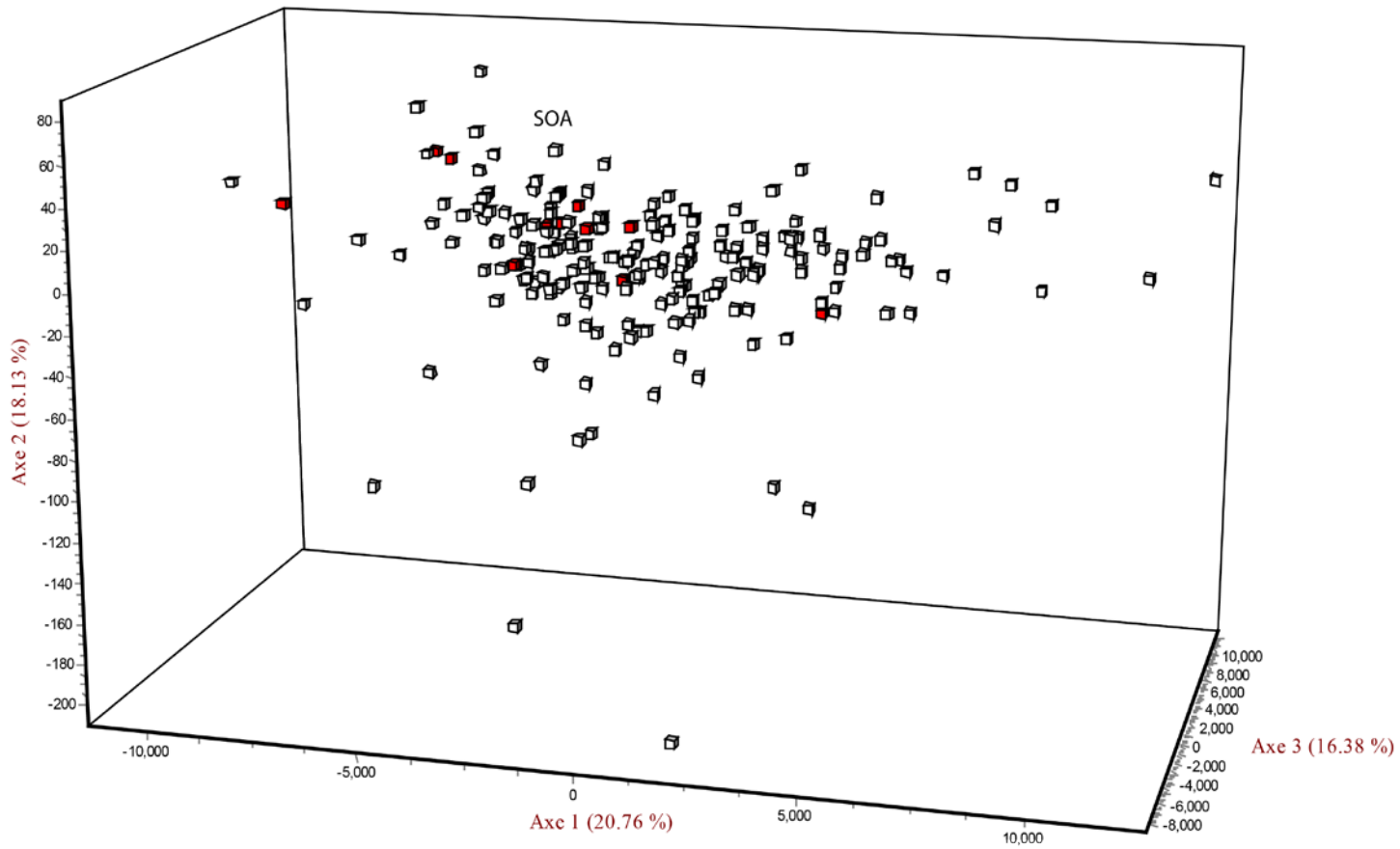
Model: No Admixture- Independent of frequencies

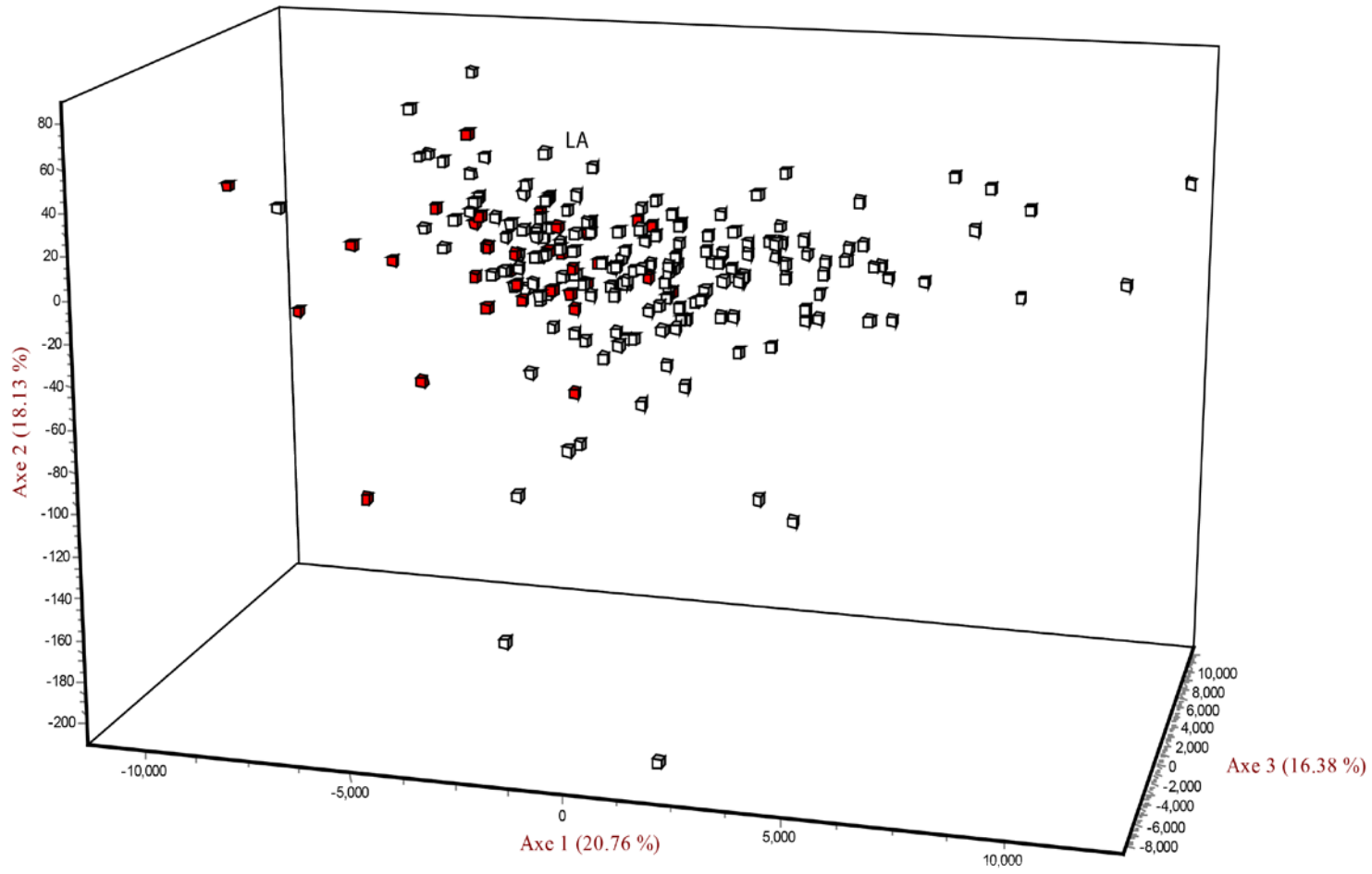
APPENDIX B

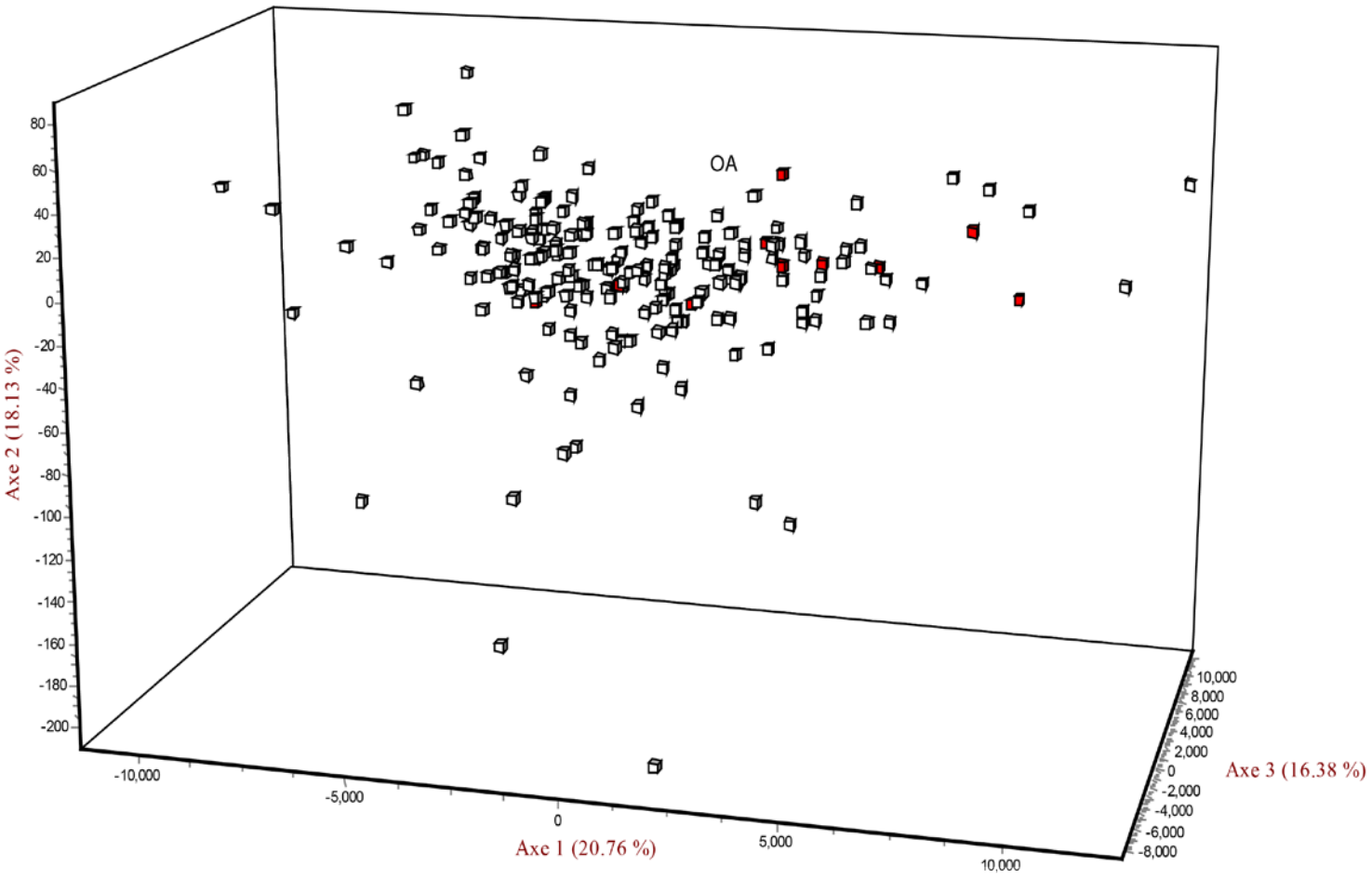
FCA'S DEPICTING LOCAL POPULATION STRUCTURE

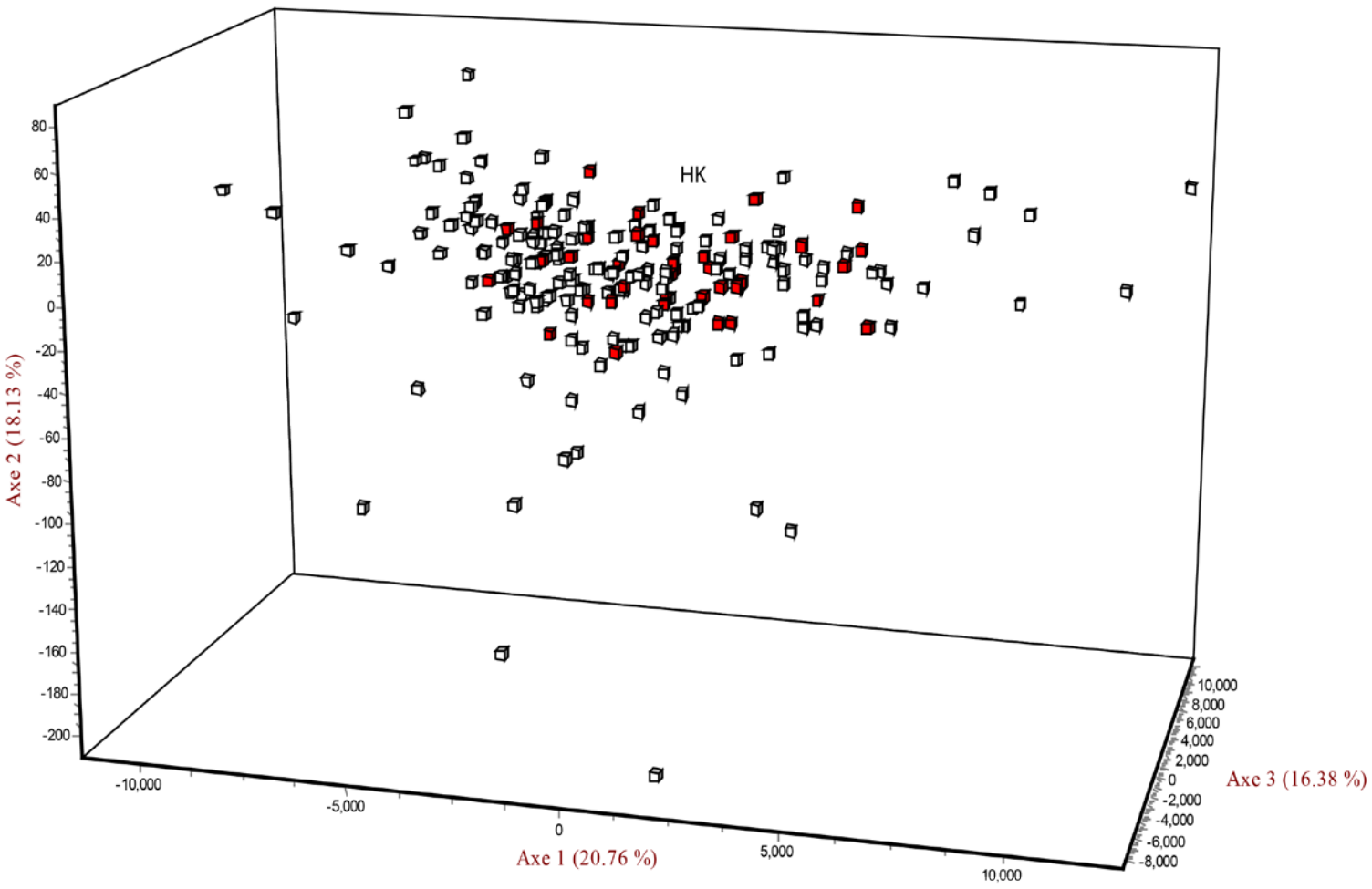


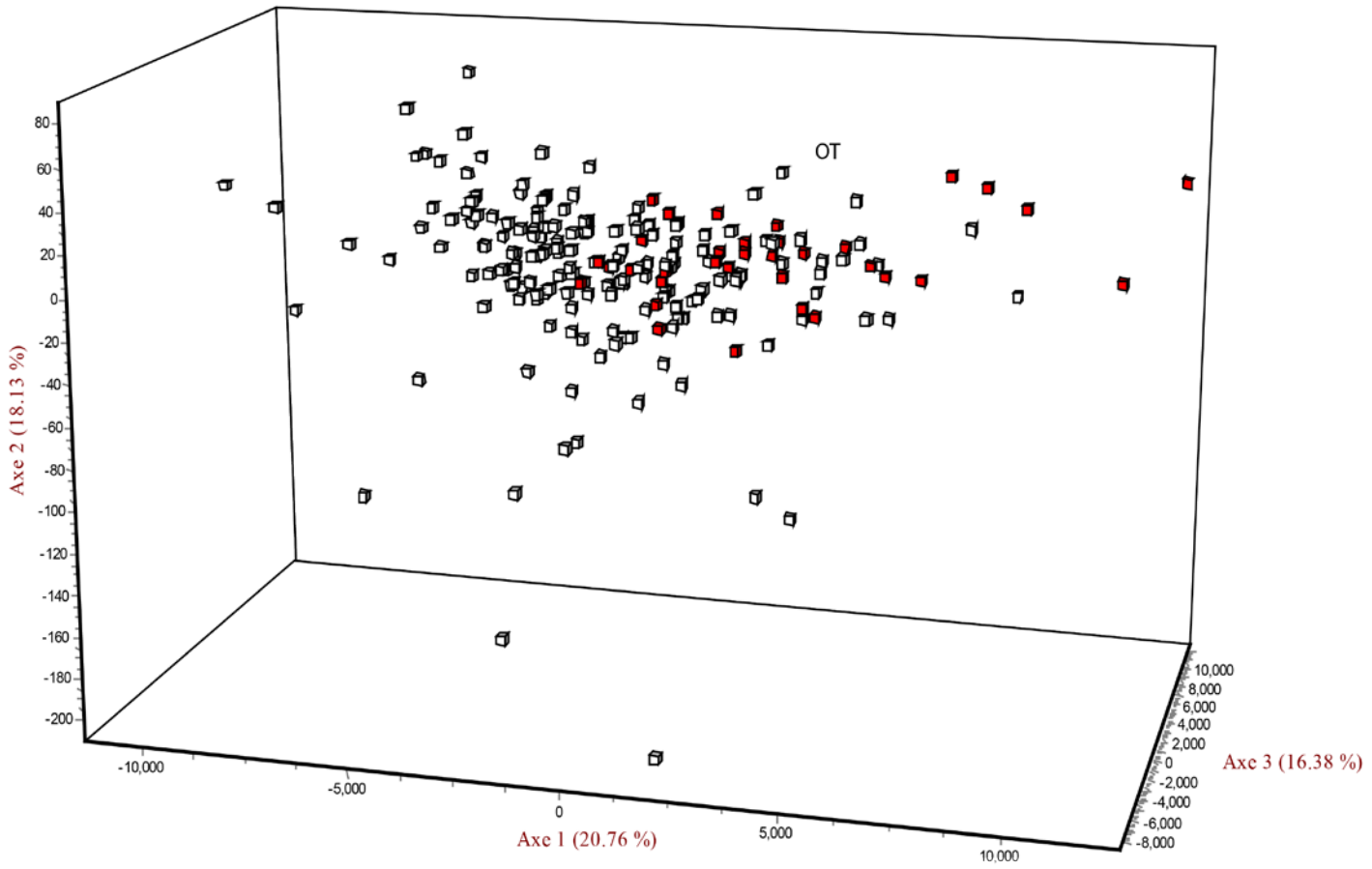

LC
Axe 2 (18.13 %)
80
60
40
20
0
-20
-40
-60
-80
-100
-120
-140
-160
-180
-200
-10,000
-5,000
0
5,000
10,000
Axe 1 (20.76 %)
10,000
8,000
6,000
4,000
2,000
0
-2,000
-4,000
-6,000
-8,000
Axe 3 (16.38 %)

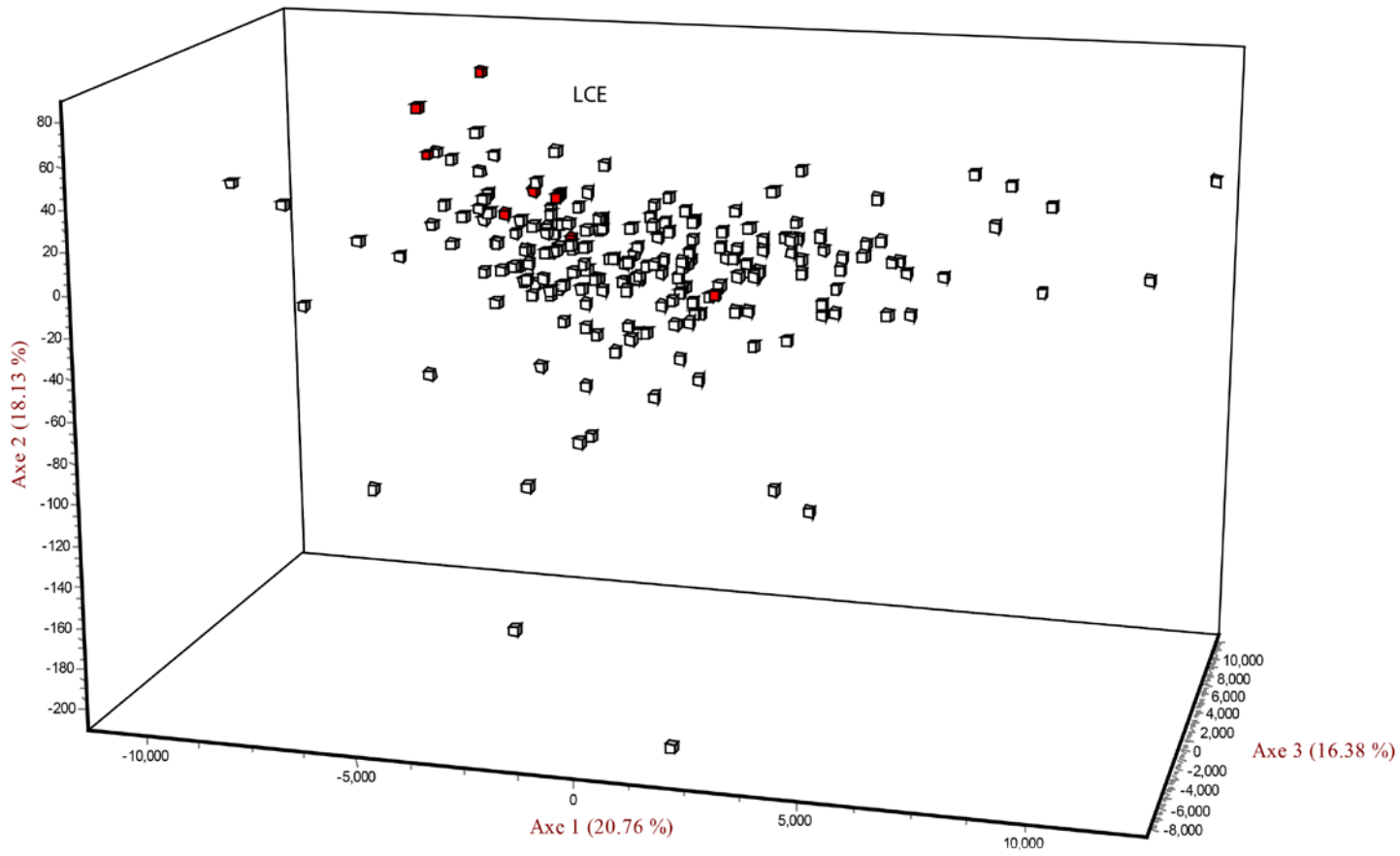

SOA
Axe 2 (18.13 %)
Axe 1 (20.76 %)
Axe 3 (16.38 %)
80
60
40
20
0
-20
-40
-60
-80
-100
-120
-140
-160
-180
-200
-10,000
-5,000
0
5,000
10,000
10,000
8,000
6,000
4,000
2,000
0
-2,000
-4,000
-6,000
-8,000

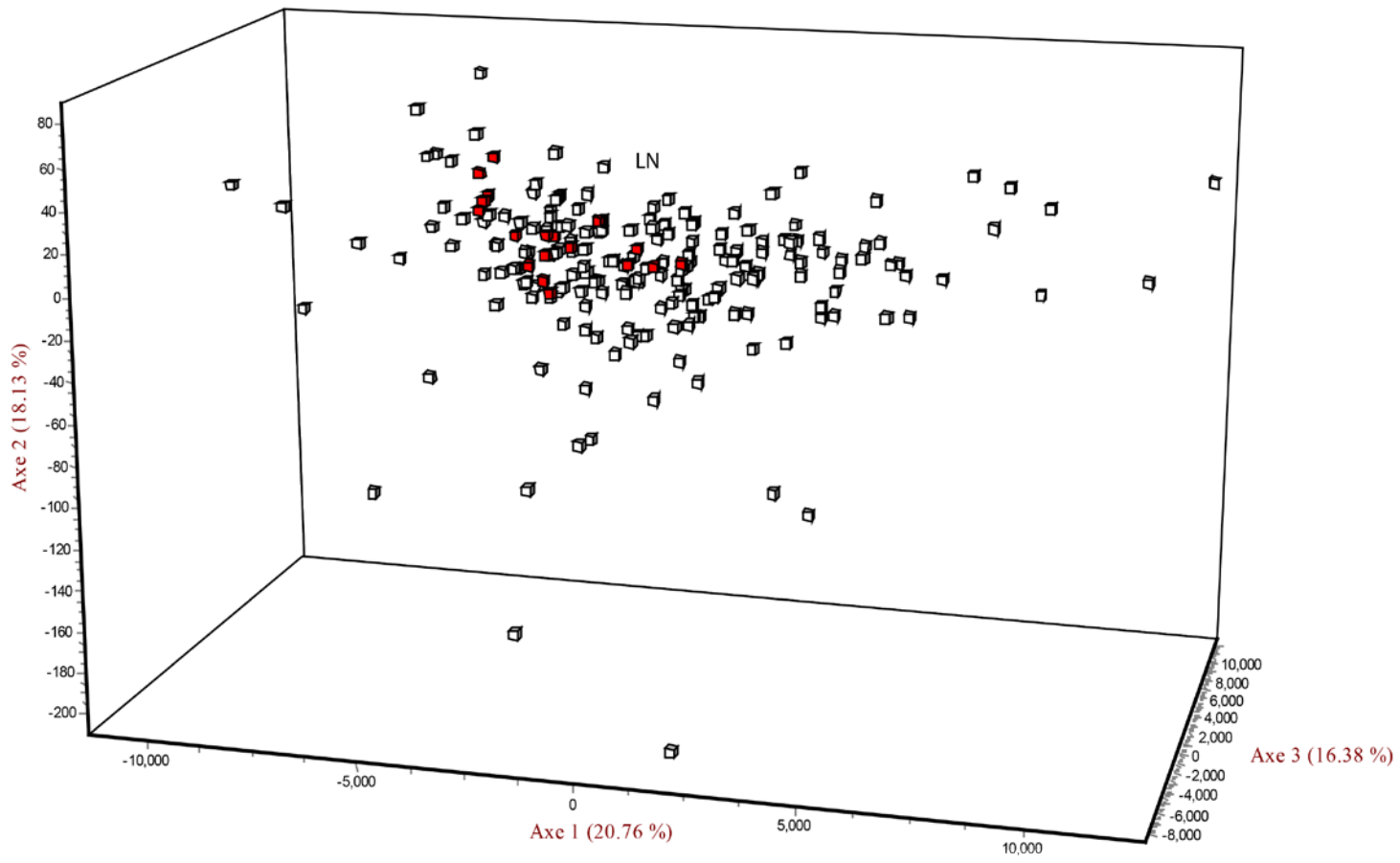

LA
Axe 2 (18.13 %)
80
60
40
20
0
-20
-40
-60
-80
-100
-120
-140
-160
-180
-200
-10,000
-5,000
0
5,000
10,000
Axe 1 (20.76 %)
10,000
8,000
6,000
4,000
2,000
0
-2,000
-4,000
-6,000
-8,000
Axe 3 (16.38 %)

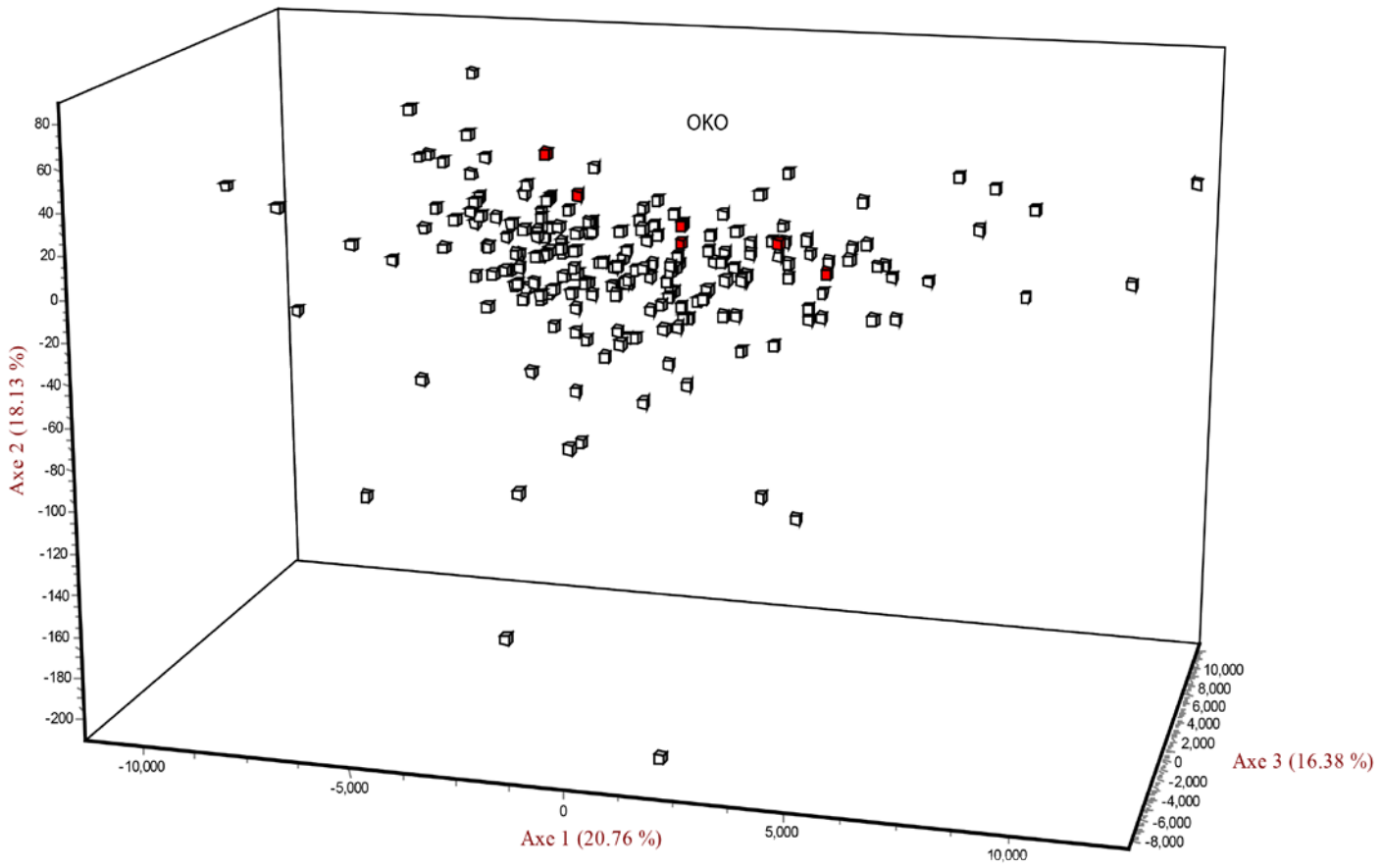

OA
Axe 2 (18.13 %)
80
60
40
20
0
-20
-40
-60
-80
-100
-120
-140
-160
-180
-200
-10,000
-5,000
0
5,000
10,000
Axe 1 (20.76 %)
10,000
8,000
6,000
4,000
2,000
0
-2,000
-4,000
-6,000
-8,000
Axe 3 (16.38 %)


HK
Axe 2 (18.13 %)
Axe 1 (20.76 %)
Axe 3 (16.38 %)
80
60
40
20
0
-20
-40
-60
-80
-100
-120
-140
-160
-180
-200
-10,000
-5,000
0
5,000
10,000
10,000
8,000
6,000
4,000
2,000
0
-2,000
-4,000
-6,000
-8,000


OT
Axe 2 (18.13 %)
80
60
40
20
0
-20
-40
-60
-80
-100
-120
-140
-160
-180
-200
-10,000
-5,000
0
5,000
10,000
Axe 1 (20.76 %)
10,000
8,000
6,000
4,000
2,000
0
-2,000
-4,000
-6,000
-8,000
Axe 3 (16.38 %)


LCE
Axe 2 (18.13 %)
Axe 1 (20.76 %)
Axe 3 (16.38 %)
80
60
40
20
0
-20
-40
-60
-80
-100
-120
-140
-160
-180
-200
-10,000
-5,000
0
5,000
10,000
10,000
8,000
6,000
4,000
2,000
0
-2,000
-4,000
-6,000
-8,000


LN
Axe 2 (18.13 %)
Axe 1 (20.76 %)
Axe 3 (16.38 %)
80
60
40
20
0
-20
-40
-60
-80
-100
-120
-140
-160
-180
-200
-10,000
-5,000
0
5,000
10,000
10,000
8,000
6,000
4,000
2,000
0
-2,000
-4,000
-6,000
-8,000


OKO
Axe 2 (18.13 %)
80
60
40
20
0
-20
-40
-60
-80
-100
-120
-140
-160
-180
-200
-10,000
-5,000
0
5,000
10,000
Axe 1 (20.76 %)
10,000
8,000
6,000
4,000
2,000
0
-2,000
-4,000
-6,000
-8,000
Axe 3 (16.38 %)

VITA

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